Welfare of horses transported to slaughter in Canada and Iceland: assessment of welfare issues and associated risk factors

BY

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A Thesis Submitted to the Graduate Faculty in Partial Fulfillment of the Requirements for the Degree of

DOCTOR OF PHILOSOPHY

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Abstract

The welfare of horses transported for slaughter is a growing concern in several countries, including Canada, Iceland and the United States of America (USA). Slaughter of horses involves transportation of horses to a regulated facility and slaughter procedures such as lairage and stunning. The main objective of this study was to identify welfare issues and associated risk factors, particularly those associated with transportation.

A welfare assessment protocol was developed to identify pertinent welfare issues, such as injuries, dehydration and fitness for transportation. Prevalence of horses with injuries, a pre-existing clinical condition, a body condition score of less than 3 (on a scale of 5) and those in a non-ambulatory state were calculated. Skin temperature, respiration rate, blood lactate concentration, blood glucose concentration, plasma osmolality, plasma total protein concentration and packed cell volume were also measured.

Welfare assessment of horses in Iceland was undertaken before and after transportation to the slaughter plant and at slaughter. Forty six journeys lasting up to 3 hours were studied. Welfare issues identified were the prevalence of bruising and dehydration. Adults were more prone to bruising and dehydration than foals. Some horses showed signs of consciousness after stunning (1.6%) indicating ineffective stunning.

In Canada, a prospective study observed 150 truckloads of horses after transportation to a Canadian slaughter plant. Associations between risk factors and welfare outcomes were evaluated using linear regression models. Welfare issues identified were prevalence of injuries, pre-existing clinical conditions, low body condition scores, and the presence of some non-ambulatory horses. There was a significant association between journey duration and the number of horses per truckload with injuries. Signs of dehydration were identified and were associated with journey duration and season. Blood lactate concentration at slaughter indicated increased anaerobic metabolic activity, which was affected by season (summer or winter) and lairage duration.

A retrospective study was performed by collating data from all shipper certificates obtained from USDA for journeys in 2009 from the USA to equine slaughter plants in Canada. This study identified journey durations range from one hour to 105 hours.

Some injuries in horses transported for slaughter were visible at ante-mortem inspection, whereas other injuries, such as bruises were not visible until post-mortem examination. Digital infrared thermography (DT) was evaluated as a potential tool to detect bruising ante-mortem. A preliminary study to evaluate factors affecting skin temperature (as measured by DT) indicated that an outdoor environment significantly affected skin temperature measured on different regions of interest (ROI) compared with an indoor environment. However, thermal symmetry between ROIs was maintained in outdoor conditions. Using these findings, a second study was performed to evaluate the methodology to detect bruising ante-mortem. Sensitivity to detect bruising was low, possibly due to selection of horses that did not spend time in lairage (i.e. there was no equilibrium time for skin temperature to stabilise after transport).

In conclusion, in Canada, injuries and dehydration were mainly associated with journey duration, aggressive behaviour between horses and season. In Iceland, injuries and dehydration were mainly associated with age (adult or foal).

Acknowledgements

First and foremost, I would like to thank the funding agencies; The Sir James Dunn Animal Welfare Centre and The Animal Welfare Foundation of Canada, for investing in this project which was projected to improve the welfare of horses intended for slaughter.

This thesis would not have been possible without the help of the following people.

1. Dr. Michael Cockram
2. Dr. Ian Dohoo
3. Dr. Christopher B Riley
4. Dr. Pierre-Yves Daoust
5. Dr. Henrik Stryhn
6. Dr. J. Trenton McClure
7. Dr. Sveinn Ragnarsson
8. Stephane Giguere

Dr. Michael Cockram and Dr. Ian Dohoo wrote the grant proposal for the project and secured the necessary funding. Their commitment, expertise and guidance helped me to improve the thesis greatly and it was a pleasure to work with them. Dr. Christopher Riley was instrumental in my investigation to use infrared thermography as a welfare assessment tool. Dr. Pierre-Yves Daoust and Dr. Henrik Stryhn helped me to approach the project confidently. As Chair of my committee Dr. J.McClure helped me throughout for the smooth completion of the project.

Sveinn Ragnarsson was instrumental in gaining access to the slaughter plant in Iceland and Stephene Giguere was instrumental in gaining access to the slaughter plant in Canada. I am indebted to both of them.

I am grateful for the moral support and helpful discussions with the following people

1. Selvi Roy
2. Dr. Rapheal Vanderstichel
3. Adel Elghafghuf
4. Dr. Ramaswamy Chidambaram
5. Dr. Anne Pearson
6. Dr. Mary A. McNiven

I would like to thank the Physics Department, UPEI for lending me an infrared thermography camera for the pilot study on factors that affect skin temperature as measured by Digital infrared thermography.

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LIST OF ABBREVIATIONS

A	Radiating area
AFAC	Alberta Farm Animal Care
AVMA	American Veterinary Medical Association
B	Blood
BCS	Body Condition Score
BSE	Bovine Spongiform Encephalopathy
CI	Confidence interval
CFIA	Canadian Food Inspection Agency
CK	Creatine kinase
cm	Centimetre
CNBC	Consumer News and Business Channel
DC	District of Columbia
DT	Digital infrared thermographic image/Digital thermography
DEFRA	Department of Environment, Food and Rural Affairs, UK
EC	European Council
EU	European Union
EBSCO	EBSCO research database
EFSA	European Food Safety Authority
FOAIQ	Freedom of Information Act request
FAWC	Farm Animal Welfare Council
FAO	Food and Agriculture Organization
FEI	International Equestrian Federation
FLIR	FLIR systems is a manufacturing company
g	Gram
GPO	US Government printing office
h	hours
HP	Heparinised plasma
IU	International Units
K	Kelvin
kg	Kilogram
OIE	World Organisation for Animal Health
l	Litre
m	Metre
MASS	Modern Applied Statistics
Max	Maximum
Min	Minimum
mmol	Millimole
n	Sample size
NFACC	National Farm Animal Care Council

N: L	Neutrophil: Lymphocyte
n/a	Not applicable
NPV	Negative predictive value
ns	Not significant
NY	New York State
ON	Ontario Province
OR	Odds Ratio
P	Net radiated power
P	Probability
PA	Pennsylvania
PCV	Packed Cell volume
PPV	Positive predictive value
Q_1	First quadrant
Q_3	Third quadrant
Ref	Reference level
ROI	Region of interest
RTA	Right to Information request order number
S	Serum
sd	Standard deviation
SE	Standard error of the coefficient
SI	International System of Units
T_g	Black globe temperature
T_{wb}	Wet bulb temperature
T^4	Fourth power of temperature of radiator
T_c^4	Fourth power of temperature of surroundings
UPEI	University of Prince Edward Island
USA	United States of America
USDA	United States Department of Agriculture (USDA)
UK	United Kingdom
W	Average weight of the horse in kilograms
WBGT	Wet bulb globe temperature
ε	Emissivity
σ	Stefan–Boltzmann constant
%	percent

CHAPTER 1

WELFARE ISSUES ASSOCIATED WITH THE TRANSPORT AND SLAUGHTER OF HORSES

1.1. INTRODUCTION

1.1.1 Horse population and horse meat industry

One-sixth of the world's population of horses lives in Canada and the United States of America (USA). Of the total population of 59 million horses in the world in 2012, Canada had 400 thousand horses and the USA had 10 million horses (FAO statistical database, 2012a). Since 1961, the horse population in Canada and the USA has been steadily increasing (Figure 1.1), whereas the horse population in Europe has declined and the population has remained stable in Asia and Africa (FAO statistical database, 2012a). The author hypothesizes that when the population of horses is increased, the number of horses which need to be culled or euthanized may also increase (Messer, 2012).

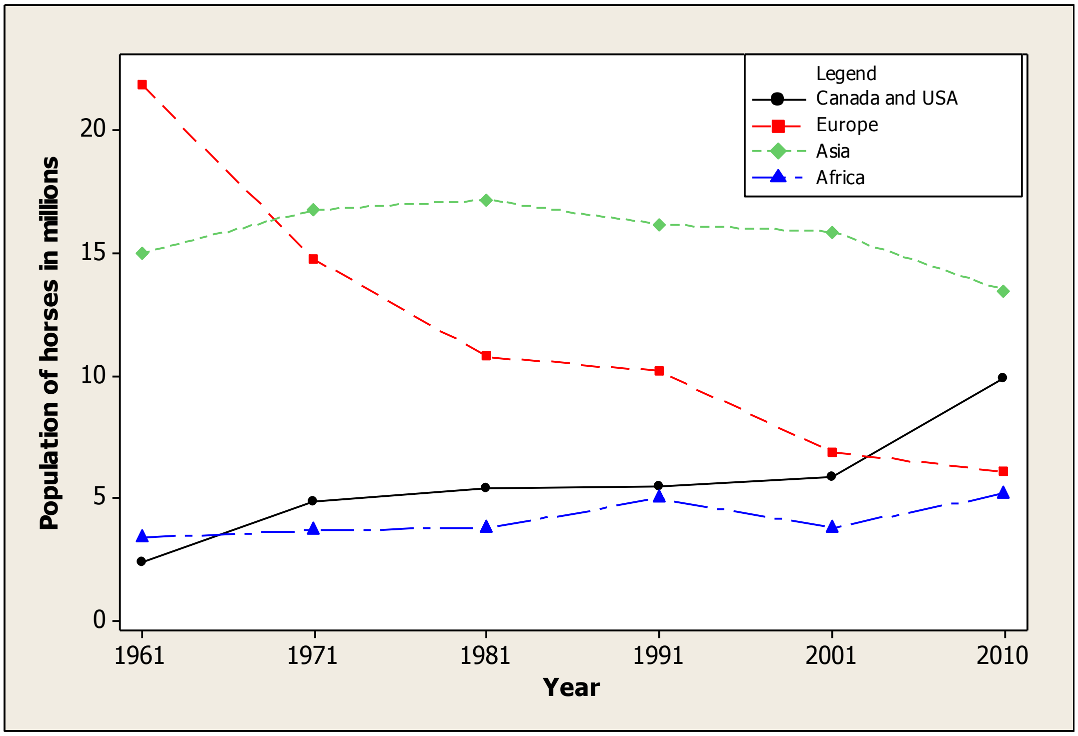

Figure 1.1: Time series graph showing the population trends of horses between 1961 and 2010 in Canada and the USA compared to Europe, Asia and Africa (FAO statistical database, 2010a).

In 2005, the horse industry made an overall contribution of $102 billion to the USA economy (American Horse Council, 2005). Similarly, the Canadian horse industry provided an annual economic contribution in excess of $19 billion to the Canadian economy (Equine Canada, 2010). In Canada, the number of horse farm operators increased by 37% from 2001 to 2006 (Statistic Canada, 2008). The 'Equine Canada' horse industry profile survey reported that the majority of horses in Canadian farms were used for sport and leisure, followed by use for breeding purposes, companionship, meat production and production of products for the pharmaceutical industries, such as pregnant mare urine production (now in decline) (Equine Canada, 2010). A similar study by Equine Canada in 2003 pointed out that there had been steady growth in horse ownership between 1996 and 2003, driven mainly by "new participants" entering the market (Equine Canada, 2003). At the time of the report, the ownership of horses was highest in the provinces of Alberta, Ontario, Saskatchewan, Manitoba and British Columbia in descending order. The majority of horses bred in Canada were Arabians, Morgans, Quarter horses or Warm blood breeds and Thoroughbreds (Equine Canada, 2003).

In addition to horses being used for recreation and performance purposes, some countries with a large equine population also slaughter horses to produce meat for human consumption or for pet food. Apart from the USA and Canada, countries with a large horse populations include China (6.8 million), Mexico (6.3 million), Brazil (5.6 million), Argentina (3.6 million) and Ethiopia (1.7 million) (FAO statistical database, 2012a). In most of these countries, once the horses are no longer needed for their initial purposes, (e.g. draught or recreational purposes) a percentage of horses are slaughtered for human consumption or inclusion in pet food (Martuzzi *et al.*, 2001). Countries which produce horse meat can use most of it for domestic consumption (e.g. Russia and China), whereas others (e.g. Canada) export it to countries where there is demand.

About 745,499 tonnes of horse meat was produced in 2008 worldwide (FAO Statistical database, 2012b) of which Canada (the tenth largest producer) produced 2.4 percent (Table 1.1). Export of horse meat contributed $60 million in 2003 and $90 million in 2008 to the Canadian economy (Statistics Canada, 2010). Only ten percent of the horse meat produced in Canada is consumed locally; the rest is shipped to countries of the European Union (Global Agricultural Information network, 2010) and Japan (Agriculture and Agri-Food Canada, 2010). Approximately, 30% of the horse meat consumed in Europe comes from Canada (Global Agricultural Information network, 2010).

Table 1.1: Distribution of the number of horses slaughtered and amount of horse meat produced by country, in 2008. The number of horses slaughtered may not correspond directly with the production of meat due to differences in yield of different carcasses according to breed size and other factors (Data compiled from FAO statistical database).

Country	No. of horses slaughtered in 2008	Production of horse meat in 2008 (tonnes) (1 tonne=1000kg)	Percentage of world production
China	1 650 000	1 98 000	26.6
Mexico	630 000	79 380	10.6
Kazakhstan	360 000	66 300	8.9
Argentina	265 000	57 000	7.6
Russian Federation	246 238	45 945	6.2
Mongolia	312 500	38 100	5.1
Kyrgyzstan	108 900	25 300	3.4
Italy	97 648	24 565	3.2
Brazil	165 200	21 500	2.9
Canada	88 000	18 000	2.4
World	4 824 610	7 35 210	

In addition to some European Union countries such as France, Italy, Belgium, and The Netherlands (Gregory, 2007), horse meat is also favoured in China, Mexico, Kazakhstan, Japan and Iceland. In Iceland, the horse population and number of horses slaughtered for meat has increased moderately in the last few decades. During this period, the horse population has increased from 30,000 to 80,000 (FAO statistical database, 1961-2009) and the number of horses slaughtered for meat has increased from 7,000 to 8,000. When compared to Canada, fewer horses are slaughtered in Iceland from the 8 slaughter plants there (Chapter 3).

The number of horses slaughtered in Canadian slaughter plants increased substantially from 2006 to 2011 (Figure 1.2). One of the explanations provided for this increase was the closure of slaughter plants in the USA that kill horses for human consumption during this period (Shames, 2011). Horses can be slaughtered for pet food in the USA; however, recent literature suggests that it is of negligible proportion (Okuma and Hellberg, 2014). It has also been suggested that over the past ten years, horse meat consumption has increased in Europe, possibly because of concerns with respect to Bovine Spongiform Encephalopathy (BSE) outbreak (Gregory, 2007). There are currently four federally approved slaughter plants in Canada (Table 1.2) that have the infrastructure for the slaughter of horses and export of the meat products.

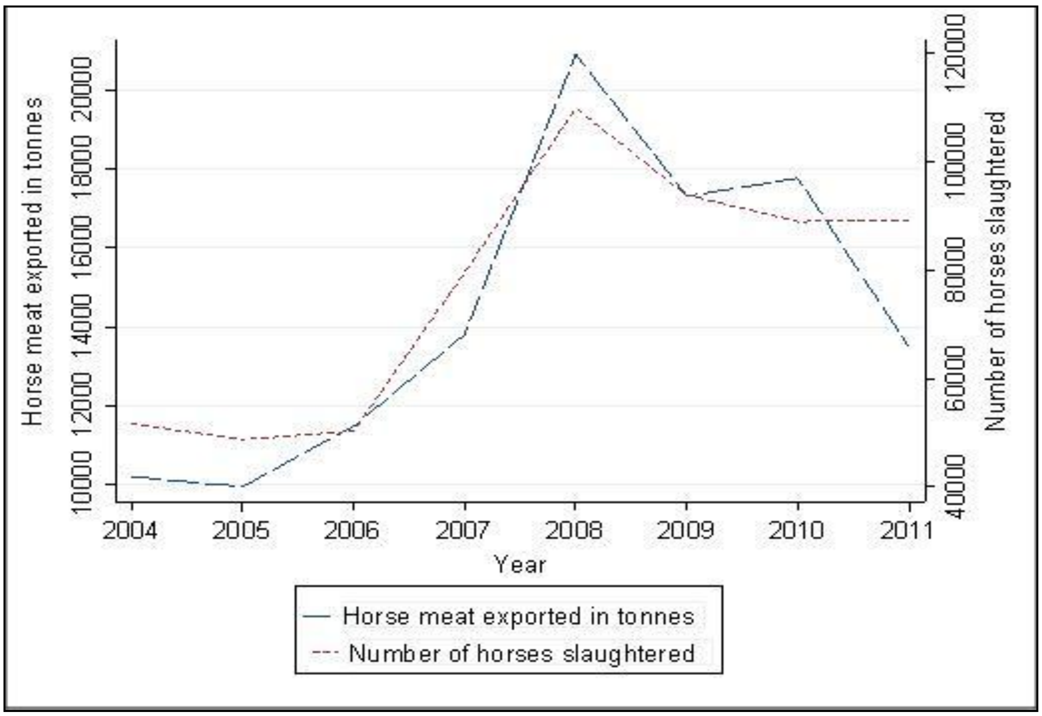

Figure 1.2: Number of horses slaughtered in Canada and corresponding weight of horse meat exported from 2004 to 2011 (Agriculture and Agri-Food Canada, 2011).

Table 1.2: Details of the four federally approved horse slaughter plants in Canada including registration number, name of operator, and the different species of animals that each plant is approved to slaughter.

Registration number	**Name of the operator**	**Species processed**
076	Viande Richelieu Inc. / Richelieu meat Inc., Québec.	Cattle, calves, horses and others
505	Les Viandes De La Petite-Nation Inc., Québec.	Cattle, calves, horses and others
506	Bouvry Export Calgary Ltd., Fort Macleod, Alberta.	Cattle, calves, sheep, lambs, horses, goats and others
657	Canadian Premium Meats Inc., Lacombe, Alberta.	Cattle, horses and others

As described above, Canada derives considerable economic benefit from the horse industry and the horse meat industry sector (Agriculture and Agri-Food Canada, 2010). Increasingly, the North American public and non-governmental organisations are showing ethical sensitivity to animal welfare issues involved in the transport and slaughter of horses for human consumption (Albert Farm Animal Care, 2008). This makes a compelling argument to examine current practices and identify any welfare issues in this industry, particularly during the transport and slaughter of horses. Identification of welfare issues and incorporating procedures to rectify or mitigate these concerns may help the industry gain public support as compassionate providers of a much needed service for unwanted horses (see section 1.1.2 below). Additionally, as exporters of horse meat to Europe for human consumption, Canadian slaughter plants also need to comply with European Union directives and the World Organisation for Animal Health (OIE)'s Terrestrial Animal Health Code regarding animal welfare and health (European Commission, 2010). Currently, there is limited scientific information available on the transport and slaughter of horses in Canada to underpin informed regulatory policies. The main aim of this project was to capture objective information regarding the welfare of horses transported for slaughter and to identify risk factors for poor welfare.

1.1.2. Unwanted horses

Unwanted horses are an emerging welfare issue facing the USA and Canadian horse industry. The term "unwanted horse" was used by the American Association of Equine Practitioners at a horse industry meeting in Washington, DC. Unwanted horses are defined as "those no longer wanted by their current owner because they are old, injured, sick, unmanageable, or fail to meet their owner's expectations" (Lenz, 2009). Unwanted horses generally include those that are aged, deemed dangerous, injured, have a history of breeding failure, and mares no longer required for the pregnant mare urine industry (North *et al.*, 2005). Human factors such as losing interest in owning a horse, change in employment or economic status, divorce, children growing up and moving away from the family residence have also contributed to this problem (The Unwanted American Horse Coalition, 2009). A utilitarian way of dealing with these unwanted horses is to send them for slaughter, so that the meat may be used for consumption in countries where there is demand. This increases the salvage value of the horse and may also keep the price of the remaining live horses higher and influence owners to retain them for longer (North *et al.*, 2005).

A vibrant horse industry along with continuous growth in the equine population in Canada and the USA has contributed to the creation of unwanted horses that have to be managed effectively and humanely. There is increasing evidence to suggest that the number of unwanted horses has increased in the USA after the closure of horse slaughter plants there (The Unwanted American Horse Coalition, 2009). There is also anecdotal evidence of abandonment of some horses by owners due to financial difficulties (Collins *et al.*, 2010; Shames, 2011). Mostly, these reports are from countries where there is a cultural taboo for horse slaughter for human consumption such as the USA (Messer, 2012). In countries where there is no horse slaughter, the disposal of unwanted horses without suffering is an important dilemma that policy makers are grappling with (Messer, 2012).

A stakeholders' conference in 2008 supported by the United States Department of Agriculture (USDA) and many horse welfare groups on 'The unwanted horse issue: What now?" made an attempt to address this issue (Heleski *et al.*, 2008). Most stakeholders at the conference agreed that a high population of horses in any country would create some percentage of unwanted horses. Lenz (2009), after studying the USDA data on unwanted horses for a few years, indicated that at least three to four percent of the population normally falls under the category of unwanted horses every year, whereas other workers argue that this figure may be as high as five to 10 percent (North *et al.*, 2005). Extrapolating a conservative four percent to the Canadian horse population suggests that this category amounts to approximately 28,000 horses every year in Canada. Some percentage of these unwanted horses may be retrained and used for other purposes, such as therapeutic riding programs and others may be euthanized by a veterinarian, donated to university animal science departments, or accommodated by rescue/retirement facilities run by non-governmental organisations (Messer, 2012). Even then, it is unclear whether all these horses can be disposed of in some way without affecting their welfare.

Currently, many unwanted horses from Canada and portion of these horses from the USA are slaughtered for human consumption and the pet food industry.

A national economic impact study estimated that 62% of the horse owners in the United States of America (USA) had an annual income of $25,000 to $75,000 (American Horse Council, 2005). The annual income level of horse owners in Canada also follows a similar pattern (60,000 to 80,000 Canadian dollars) to that of the USA (Equine Canada, 2003). It is imperative for most horse owners within this income group to have an economically viable way to dispose of unwanted horses.

Generally, the demand for horses, particularly in leisure and sporting sectors closely follows other economic trends (The Unwanted American Horse Coalition, 2009; Lenz, 2009). When the economy is doing well, the demand for “pleasure horses” increases but when the economy is not doing well, the demand decreases. This trend increases the likelihood of creating more unwanted horses during an economic downturn. The link between the general economy and unwanted horses makes a compelling argument that discontinuing slaughter could affect the welfare of some of these horses due to neglect and abandonment. The burden of disposing of horses by methods other than slaughter in an abattoir can be excessive for horse owners- particularly for owners with financial difficulties. In Canada, considering the current situation where unwanted horses can be slaughtered for human consumption and for other purposes such as pet food, the next issue to tackle is whether this operation (transport and slaughter of horses) can be undertaken with minimum distress and discomfort to the horses involved.

1.1.3. Horse slaughter: animal rights and animal welfare perspectives

The fundamental difference between an "animal welfare" and an "animal rights" perspective is regarding the real value of the animal's life. The animal welfare perspective takes a utilitarian and protectionist view of the animal's life and hence suggest that horses can be used for human consumption, as long as this activity is performed humanely without suffering (Whiting, 2007). In contrast, the animal rights perspective fundamentally disagrees with this view and considers that the worth of an animal's life is equivalent to that of a human being, and hence no animal should be slaughtered (Phillips *et al*., 2010). Even though the animal rights perspective is an empathetic thought process, whether this philosophy is ethically, politically and economically feasible is debatable (Francione and Garner, 2010).

Horse slaughter for human consumption is generally opposed by the animal rights groups and also by some animal welfare promoting groups (Whiting, 2007). These animal welfare promoting groups argue that horses are companion animals and are unlike other food animals such as pigs, cattle and sheep. The view within these groups is that horses are not bred for human consumption, particularly in the USA and Canada; hence, they should not be slaughtered for human consumption (Whiting, 2007).

Another argument by some animal welfare groups in the USA who oppose the slaughter of horses for human consumption is that "nothing significantly changed for the worse after the stoppage of slaughter in the USA" (Hazard, 2008). They say that all unwanted horses are accommodated in sanctuaries or utilized for other purposes after the stoppage of the horse slaughter in the USA (which came into effect in 2007). However, evidence suggests that there was an increase in the population of unwanted horses in the USA after this stoppage (The Unwanted American Horse Coalition, 2009). These horses are either transported out of the USA for slaughter in Mexico or Canada (Larkin, 2010; Nolen, 2008) or as anecdotal evidence suggests abandoned within the country. Therefore, the argument that unwanted horses are not an issue, when the number of horse slaughter plants decreased (Heleski *et al.*, 2008) is uncorroborated by available evidence.

The two real issues regarding unwanted horses are first, whether there are enough viable options for the horse owners to dispose of unwanted horses without compromising their welfare. Second, whether horse slaughter can be performed with acceptable welfare standards equivalent to those expected for the slaughter of other livestock, such as cattle, pigs and sheep.

There are a growing number of experts who believe that horse slaughter can be an effective way to reduce the welfare issues associated with unwanted horses. After studying the problem of unwanted horses, Whiting (2007) opined that a law banning horse slaughter should be based on scientific data and not upon cultural prejudice. If welfare assessment studies show that the slaughter of horses cannot be done humanely, or if horses during transport for slaughter are more susceptible to injuries, disease, fear and pain, because their behaviour is different from other livestock, such as cattle, sheep and pigs, then there may be a case for this ban.

1.2. ASSESSMENT OF WELFARE

There has been increasing acceptance among the scientific community and to some extent, the general public that animals are sentient beings (Duncan, 2006). In response to this acceptance, ethics based arguments from various professional communities have highlighted the need to assess animal welfare at critical points, particularly when we use them for our benefit such as food, sports, trading and draught purposes. They argue that reliable monitoring systems for assessing welfare status and evaluating risks are necessary in order to accommodate these growing societal concerns and market demands (Blokhuis *et al.*, 2003).

Any welfare assessment tool developed should help veterinarians, animal scientists, producers, transporters, auditors, government agencies, quality assurance managers and others to quantify a welfare problem. By doing this, mitigation strategies can be developed for better welfare practices. Welfare assessment tools should be practical and should help evaluate the changes in welfare longitudinally over time.

In general, welfare assessment highlights the importance of animal welfare and provides a baseline welfare status of the population studied. Thereafter, it helps improve the welfare status of animals in a gradual way (Sørensen and Fraser, 2010), as objective comparisons can be made to the baseline welfare status. It also provides an opportunity to study risk factors associated with each welfare problem. Figure 1.3 shows some of the risk factors associated with the long distance transport of horses, which can be environmental, human or animal based.

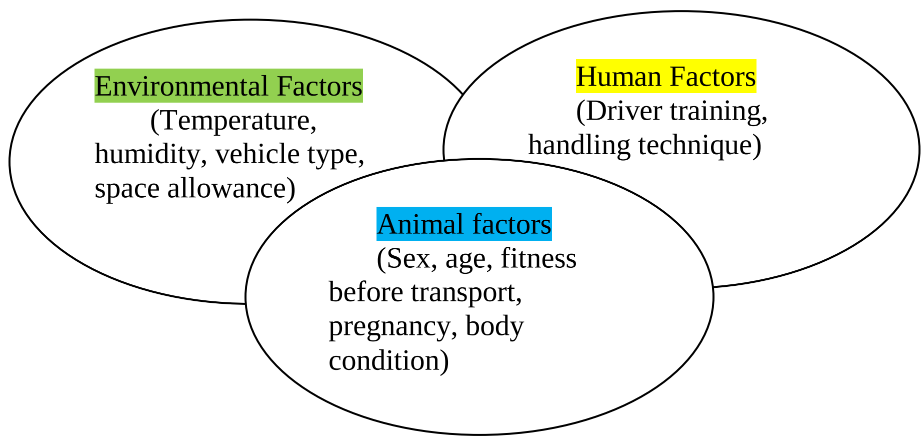

Figure 1.3: Examples of environmental, human or animal-based factors that can affect the welfare of horses, particularly when transported for long distances.

One starting point for the development of a welfare assessment protocol is an analysis of the Farm Animal Welfare Council's (FAWC) five freedoms (FAWC, 1979) and critically examining how transport procedures impact upon them. As discussed by Cockram (2007), each of FAWC's five freedoms could potentially be influenced by transport as follows:

1. "Freedom from thirst, hunger and malnutrition: water and feed restriction during a journey and lairage and changes in diet.
2. Freedom from discomfort: thermal and physical discomfort as a consequence of extremes of temperature, inadequate ventilation and space, vibration, acceleration and motion.
3. Freedom from pain, injury and disease: handling, impacts associated with motion, interactions between animals and infection.
4. Freedom to express normal behaviour: behavioural restriction due to confinement, motion and social disruption.

5. Freedom from fear and distress: handling, confinement and exposure to novel stimuli".

Apart from FAWC's five freedoms approach, two other commonly used definitions of animal welfare are the "welfare of an animal is its state as regards its attempts to cope with its environment" (Broom, 1988) and "fit and feeling good" (Webster *et al.*, 2004). The first definition focuses on the physiological mechanisms of the body in response to a stressor, whereas the second definition focuses on the mental status of the animal along with health. The FAWC's five freedoms incorporate both these definitions in an indirect way. Freedom from hunger, thirst, discomfort, pain, injury, disease, fear and distress could help the animal to cope better, increase its fitness and provide an opportunity to feel good.

In the last two decades, the scientific community has discussed and argued over many ways to assess welfare. Some have developed welfare assessment protocols using animal-based assessment variables (also called outcome-based) while others have used management-based assessment variables (also called resource-based or risk based), and some have used both types of variables in combination (Scott *et al*., 2003). Currently, most assessment protocols use a combination of animal-based and resource-based variables.

***Animal-based or outcome-based assessments*:** Animal-based assessments are preferred by some specialists who take the view that the animal itself is the primary stakeholder of its welfare, and hence animal-based variables should be directly observed or quantified by the welfare assessor. They provide a more direct assessment of welfare than resource-based assessments (Pritchard *et al.*, 2005). However, critics might argue that resource-based assessments are more valuable as they can be measured reliably and consistently and help identify risk factors, which affect welfare. Examples of some variables used in animal-based assessments are body condition score (BCS), lameness score and coat condition score (Sevi, 2009) along with physiological variables such as blood lactate concentration, total plasma protein concentration, and plasma osmolality (Stull and Rodiek, 2002). Behaviour based variables are also an important part of animal-based assessments. Behaviour variables can focus on expressions of positive outcomes such as play behaviour or negative outcomes such as behaviour associated with fatigue, frustration or aggression (Yeates and Main, 2008). The use of animal-based variables to assess the welfare of farm animals has increased in recent years. However, reliability (repeatability, reproducibility and robustness) and validity (accuracy and precision) have been a problem with animal-based assessment variables (EFSA Panel on Animal Health and Welfare, 2012).

***Management practices based or resource-based assessments*:** These variables are relatively easier to measure than animal-based variables because of fewer issues regarding objectivity and repeatability (Pritchard *et al.*, 2005). Mostly, these variables indicate the risk of a welfare problem occurring rather than assessing the welfare of the animal directly (Rousing *et al.*, 2001). For example, space allowance is a resource-based variable which can be measured; however, any value in terms of space allowance (high or low) may not be directly correlated with the welfare status of animals kept at that space allowance. Nevertheless, this type of assessment variable has been extensively used in guidelines and regulations. For instance, space allowance for adult horses during transport specified in the European Union regulation (EC no. 1/2001) is 1.75 m^2 per horse

(The European Council, 2005) whereas the Canadian guideline prescribed by the National Farm Animal Care Council (NFACC, 1998) is 1.4 m^2 per adult horse.

Knowles *et al.* (2010) and Collins *et al.* (2000) produced evidence to infer that a reduction in the space allowance below the NFACC prescribed recommendation increases the risk of injuries and the occurrence of behaviour, such as falling and slipping during transport. Therefore, horses transported with a space allowance below the recommended levels could be recorded by welfare auditors as an indication of poor resource provision as the risk for injury is increased. Another example of usage of a resource-based variable for regulation is the OIE's welfare standard for slaughter, which has developed a list of practices that should not be allowed in a slaughter plant (OIE, 2012). For example, one of the recommendations is that an electric goad/prod should not be used on horses intended for slaughter. Usage of an electric goad/prod while handling horses during slaughter is an example of a resource-based variable that could be incorporated in a welfare assessment protocol by welfare auditors.

1.2.1. Criteria for selection of welfare assessment variables

Selection of multiple variables for welfare assessment is an important first step for any researcher, farmer, producer or other stakeholder who is interested in assessing animal welfare. Selection of such variables for assessment of welfare is often difficult because of the subjective nature of many welfare issues such as distress, discomfort, hunger, thirst, fatigue and pain. Nonetheless, some variables have been successfully used by researchers to assess welfare (Shimmura *et al*., 2011). These variables could be a measurement of a husbandry input such as presence of food and water or space allowance provided, or may involve measurement of a health-based variable by observation of the animal or animals directly.

One critical factor to be considered while selecting a welfare assessment variable is to understand how it behaves over time in response to a particular management procedure (e.g. response of animals to transport). If an assessment needs to be developed to understand the stressors associated with transport of horses, a variable X could be chosen as a marker. This marker (X) would hopefully remain unchanged for a particular period of transport and then start increasing or decreasing consistently to a ceiling point which is lower or higher than the starting value (Figure 1.4). If the variable chosen steadily increased or decreased, it may be more difficult to determine a particular limit for journey duration.

Figure 1.4: A hypothetical model of relationship between a physiological variable and a risk factor. As an example, journey duration is chosen as the risk factor (Cockram, 2007).

Some variables are appropriate to determine short-term stress while others are more suitable for long-term stress. For example, cortisol concentration could be selected if the focus of investigation was a short-term stressor, whereas if the stressor of interest is long-term, then an assessment variable such as blood lactate concentration (influenced by the amount of muscle activity), osmolality or body condition (depending on the stressor) may be more appropriate (Broom, 1998)

Variables chosen for welfare assessment protocols should help reduce the subjectivity of assessment and increase objectivity, thereby improving the validity and reliability of such an assessment. Standards, guidelines and regulations developed to assess welfare have often been subjective and hence allow lenient interpretation (Grandin, 2010c). For example, to prevent overcrowding and overloading issues during transport of animals, the Health of Animal's Regulation of Government of Canada says "No person shall transport or cause to be transported any animal in any railway car, motor vehicle, aircraft, vessel, crate or container that is crowded to such an extent as to be likely to cause injury or undue suffering to any animal therein" (Canada Department of Justice, 1990). An objective way of addressing overcrowding and overloading is by specifying space allowance or stocking density. However, it could be argued that this regulation identifies overcrowding in two situations: one in which injury or other suffering is observed as an obvious consequence of the low space allowance and, another as a situation that was likely to have caused any observed injury or suffering, i.e. it is outcome based. The difficulty with a proscriptive numerical space allowance is that it will not be appropriate in all circumstances. Nonetheless, it is possible to proscribe a guideline with minimum space allowance for farm animals that the farming industry can use as a minimum standard.

Clear and practically measurable variables will improve consistency among auditors leading to better inter-observer reliability. Training of auditors on measuring each variable also improves inter-observer reliability (Kaler *et al.*, 2009). The assessment protocol developed by Grandin (1998) for animal handling and stunning in slaughter plants follows these principles. In this assessment, objective measures such as the number of animals which needed more than one use of the captive bolt for stunning was used to assess stunning efficiency (Grandin, 1998).

1.2.2. Health variables

Health variables are animal-based variables that may provide valuable insight on the welfare status of the animal. Health variables which are commonly used are body condition score (Pritchard *et al.*, 2005; Whiting *et al.*, 2005; Roche *et al.*, 2009), coat condition, presence of injuries, eye or vision abnormalities, body swellings, hoof abnormalities and gait abnormalities (Pritchard *et al.*, 2005). Body condition score, coat condition and hoof abnormalities provide insight about the past long-term health and possibly welfare status of the animal (Pritchard *et al*, 2005). The body condition score of the animal is an indicator of any chronic energy deficit or any chronic disease conditions. Other variables such as the number of injuries, gait abnormality and body swellings provide insight on the current state of welfare and are particularly important while assessing welfare regarding short-term managemental procedures, such as transport (Grandin, 2010c).

Transportation of horses has been considered to be a predisposing factor to respiratory disease (Oikawa *et al.*, 2004). Some signs of respiratory disease can be observed through the frequency and intensity of cough and nasal discharge and they can be used as health related variables. Dehydration affects the circulatory system which in turn can be assessed using clinical signs, such as change in pulse rate and quality, heart rate and capillary refill time (Pritchard *et al.*, 2006). However, these clinical signs may not be specific for dehydration and can also indicate other health issues (Pritchard *et al*., 2008). Moreover, some of these variables such as pulse quality may need specific training and can be done only by trained and experienced people, such as veterinarians. Therefore, health variables can be an important part of any welfare assessment protocol depending on the expertise of the assessor.

Identifying morbid animals is an important part of any welfare assessment protocol, particularly when the transport of animals is the focus of interest. Morbid animals can be identified by including specific behavioural and clinical variables in the assessment protocol. Fitness problems have been identified as a significant welfare issue in horses intended for slaughter in North America and Europe (Grandin *et al.*, 1998; Grandin *et al.*, 1999; Marlin *et al.*, 2011). Horses with fitness problems due to previous disease and injuries, can endure pain and suffering due to several stressful environmental and management factors during transport. Hence, evaluating animals for signs of pain (chronic or acute) before transport using behaviour based tools, such as low head carriage, dilated nostrils, fixed lower jaw (Ashley *et al.*, 2005) and apathy (Burn *et al.*, 2010) can be helpful to segregate unfit horses. Including a pain assessment tool as part of a pre-transport evaluation will help identify horses suffering from pain (Roy *et al.*, 2010) which in turn could help to make the decision to avoid transport for these animals.

1.2.3. Behaviour variables

Animal behaviour is described as everything that the animal does, in response to external stimuli in the environment and also in response to internal stimuli such as disease, hunger, thirst and pain (Manning and Dawkins, 1998). Some authors give more importance to the perception by the animal of its environment than to the actual environmental stimuli themselves. Pritchard *et al*. (2005) described behaviour as the expression of an animal's perception of its environment, and how it interacts with it. External stimuli, such as noise, vibration and human handling that occur during transport and slaughter can affect how the neuro-endocrine system responds (von Borell, 2001), which in turn can play a role in the behaviour of the animal (Yousef, 1988). In addition, if an animal has a negative perception of its environment (e.g. previous negative experience during transport), there is more chance of it reacting with a fight or flight response (Popescu and Diugan, 2012). Therefore, the behaviour of an animal can be used as an effective tool to assess welfare (Beaver, 2010).

In order to use behaviour as an assessment variable, a good understanding of species-specific behaviour is important. In the case of horses destined for slaughter, observing behaviour during transportation and slaughter can provide meaningful information about the animals' welfare status (Grandin, 2010b). It is essential to capture behaviours that indicate aversion, aggression and difficulty in coping (Broom, 2005). Some of these behaviours could be reluctance to move forward, freezing (reluctance to move due to fear), backing off, running away, vocalizing and biting behaviour (Broom, 2007). Having data on baseline values for these specific behaviours for horses could help create benchmarks. For example, the frequency of biting behaviour in the lairage (for a particular time period) may provide a benchmark level, to determine which management strategies are effective in reducing the frequency of biting, which has been associated with the prevalence of wounds in slaughter horses.

Unpleasant sensations or feelings due to starvation (hunger) and dehydration (thirst) can be expressed by animals as behaviour (increased motivation to eat and drink) and hence in some circumstances these behavioural expressions, such as eating or drinking, can be quantified and used as assessments of these feelings. To understand the effect of feed restriction in animals, it is easier to use behaviour variables to assess welfare (Appleby and Lawrence, 1987) rather than measuring physiological indicators for hunger.

Some of the common behaviour states or events which have been quantified to assess welfare in previous studies are;

- The frequency and duration of behaviours that may indicate pleasure (play behaviour), fear, discomfort and social disturbance (Cameron *et al.*, 2008; Hockenhull and Creighton, 2012).
- The duration of normal behaviours such as eating and drinking so that evaluation can be performed for any abnormal increase or decrease in time spent (Ray and Roubicek, 1971).
- The frequency of behaviours which indicate aversion such as freezing and backing off (Fraser, 2010).
- The emergence of any redirected behaviours or displacement behaviour or stereotypic behaviour (Cooper and Albentosa, 2005)

An extensive study involving many countries observing equines managed under different welfare scenarios showed a significant relationship between specific behaviour and conditions that indicate poor welfare (Burn *et al.*, 2010). This study established that behaviour measures of unresponsiveness to human approach and other stimuli were associated with poor body condition, abnormal mucous membrane colour, faecal soiling, eye abnormalities and injuries.

1.2.3.1. Assessment of fear

Fear is defined as the “reaction to the perception of actual danger” (Forkman *et al.*, 2007) and hence expressed as a change in behaviour that could be used for assessment of welfare. Fear in horses during transport and slaughter could be due to an innate response (isolation fear), novelty, learned negative experiences and looking at or sensing the fear of others (Gregory, 2004). Fear negatively affects horses, particularly those that are not habituated or acclimatized enough with experiences during transport. The processes associated with slaughter generally comprise events such as novel and noisy conditions during lairage and stunning, which creates fear in the horses. Some other factors of transport and slaughter that can induce fear are inappropriate handling during transport and slaughter, unsuitable flooring in the slaughter plant or transport vehicles which can be slippery, and inappropriate lighting arrangements (Grandin, 2010a).

Even though habituation can reduce the magnitude of the fear response during transport, domestic horses respond to perceived threats and novelty in much the same way as their wild ancestors (Christensen *et al.*, 2005). This is because the horse's sensory system (vision, hearing, touch and smell) may have been adapted to facilitate early detection of danger (Saslow, 2002). Studies on horses have shown that three of these sensory systems (visual, olfactory and auditory) responded to novel stimulus (predator odour) and are associated with negative emotive states and changes in behaviour such as sniffing and vigilance (Christensen and Rundgren, 2008).

An ethogram (catalogue of behaviours typically exhibited by an animal) recorded during transport of horses, indicated that vocalisation, kicking, pawing, stamping, defecating and urination could be associated with fear (Waran *et al.*, 1996). Even though defecation and urination are normal physiological functions, an increase in frequency of these behaviours indicate fear and hence could be used as an indicator of negative emotional state. Quantitative measurement of these behaviours can be used to assess welfare.

1.2.3.2. Assessment of discomfort

Transport and slaughter procedures of horses, if not managed carefully, have a high risk of causing discomfort. Thermal and physical discomfort as a consequence of extremes of temperature, inadequate ventilation and space, vibration, acceleration and motion (Cockram, 2007) are recorded during the transport of food animals. Discomfort can be caused due to hunger and thirst, particularly during long distance transport (with high stocking density without adequate free access to food and water). Measuring behaviours or events such as slips, falls, stumbles and collisions of horses with each other or with any part of the vehicle (Knowles *et al.*, 2010), can provide indirect information about the levels of discomfort that the animal may be experiencing. Some other behavioural indicators of discomfort previously studied during transport of horses are vocalization, attempts to escape, kicking and struggling (Fazio and Ferlazzo, 2003). Observation for these behaviours in the lairage could also be used to identify and quantify discomfort. Anecdotal incidences of increased aggressive behaviour due to hunger and thirst have been reported in horses (Houpt, 1981).

1.2.3.3. Assessment of social disturbance

Isolation from other horses, grouping with unfamiliar horses and mixing of sexes during transport can all be risk factors for poor welfare by creating social disturbance, sometimes causing injuries or minor stressful situations. Isolation during transport can induce fear behaviour such as pawing and sniffing in young inexperienced horses and reduced feeding behaviour (Kay and Hall, 2009). Knowles *et al.* (2010) reported that when semi-feral ponies were transported in groups of four (small group size), the level of aggression was less than in groups of eight (large group size). In this study, apart from group size, stocking density also had a significant association with aggressive behaviours, such as bites, kicks, snaps and ears kept facing backwards. When stocking density was increased, the incidence of aggressive behaviour also increased. Stallions can be more aggressive than geldings and mares and hence mixing of sexes during transport should be avoided. In order to assess social disturbance, recording behaviours which indicate aggression among horses (kicking and biting), and behaviour which indicates fear (pawing, sniffing etc) are potentially useful variables.

1.2.4. Physiological variables

When animals are subjected to a stressor, the central nervous system and endocrine system interact and the animal responds to the stressful stimulus (von Borell, 2001) in physiological and in behavioural terms. The endocrine response can be expressed in the physiological profile of the animal by changes in hormonal concentrations, such as cortisol and adrenocorticotrophic hormone. Cortisol concentration (in serum/plasma, feces or saliva), adrenaline or epinephrine concentration (serum or plasma), noradrenaline or norepinephrine concentration (von Borell, 2001), beta endorphin concentration (Fazio *et al.*, 2008; Micera *et al.*, 2010), adrenocorticotrophic hormone concentration (Fazio *et al.*, 2008) and total and free iodothyronines concentrations (Fazio *et al.*, 2009) have been used to assess welfare in horses.

Metabolic, haematological and biochemical profiles can also show significant changes depending on the magnitude and duration of the stressful event. For example, if the transport of horses is considered as the potential stressful event, elevation in total plasma protein concentration (Stull and Rodiek, 2002), blood lactate concentration (Stull and Rodiek, 2000; Stull and Rodiek, 2002) osmolality and creatine kinase activity has been reported. Apart from endocrine and haematological profiles, simple physiological health variables, such as rectal temperature and heart rate have also been used to assess welfare in horses (Fazio *et al.*, 2009).

The relationship between physiological variables (particularly haematological values) and welfare status may not always be straightforward. Physiological variables respond to external and internal stimuli to maintain homeostasis and may not always indicate pathology or worsening health and welfare status. However, many of these non-consequential variations can be segregated from pathology by application of appropriate study designs and analytical controls, so that abnormal values can be brought to light while studying these issues. Several previous studies have used physiological variables to assess welfare of horses; some of the most relevant variables associated with transport are described in Table 1.3 and Table 1.4. Most physiological variables offer a quantitative continuous value which indirectly provides some information about the welfare status of the animal.

The welfare of transported horses is considered to be compromised when they undergo negative feelings, such as fear, fatigue, motion sickness and discomfort. Feed restriction and reduced water availability during transport can lead negative emotional response. Apart from this psychological component of stress, transported animals could also suffer from ill health, such as respiratory disease or suffer from environmental stressors such as heat or cold stress. In all the above scenarios, changes can occur in the physiology of the horse being transported and hence these measurements can be used to assess welfare indirectly. Whenever physiological variables are to be interpreted or used to assess welfare, it is important to ascertain the basal level and how it fluctuates over time (Broom, 1995).

Table 1.3: Some physiological variables used by previous studies to assess welfare, which could also be used to assess the welfare of horses during transport.

Welfare issue	Physiological variable (Plasma concentration)	References for horses
Nutrient energy deprivation	Beta hydroxybutyrate	Rose and Sampson, 1982; Forhead *et al.*, 1995; Sticker *et al.*, 1995.
	Glucose	White *et al.*,1991; Forhead *et al.*, 1995; Stull and Rodiek, 2000; Stull and Rodiek, 2002; Oikawa *et al.*, 2004; Werner and Gallo, 2008.
	Urea	Forhead *et al.*, 1995; Sticker *et al.*, 1995
Dehydration	Osmolality	Friend, 2000; Pritchard *et al.*, 2006; Iacono *et al.*, 2007; Mejdell *et al.*, 2005.
	Total protein	Forhead *et al.*, 1995; Stull and Rodiek, 2002.
	Albumin	Forhead *et al.*, 1995., Friend, 2000
	PCV†	Stull and Rodiek, 2002; Werner and Gallo, 2008.
Physical exertion/ fatigue	Creatine kinase activity	Stull and Rodiek, 2000.
	Lactate	Stull and Rodiek, 2000; Stull and Rodiek, 2002; Werner and Gallo, 2008.
Transport stress	Cortisol	Stull and Rodiek, 2002; Forhead *et al.*, 1995; White *et al.*, 1991; Fazio *et al.*, 2008; Stull and Rodiek, 2000; Werner and Gallo, 2008.
	Beta endorphin	Fazio *et al.*, 2008.

†PCV measured from whole blood

Table 1.4: Reference range of physiological variables for healthy horses (Kaneko *et al.*, 1997) which can be used to indirectly assess welfare in horses and the likely response of these variables to transportation.

Physiological variable	SI units	Response to transport
PCV	32-53 %	↑ (Fazio and Ferlazzo, 2003)
Glucose (S, Plasma, HP)	4.16-6.39 mmol/litre	↑ (Stull and Rodiek, 2000)
Total protein (S)	52-79 g/litre	↑ (Friend, 2000)
Albumin (S)	23-39 g/litre	↑ (Friend, 2000)
Lactate (B)	1.11-1.78 mmol/litre	↑ (Stull and Rodiek, 2000)
Creatine kinase (S, HP)	7.7-18.1 IU/litre	↑ (Stull and Rodiek, 2000)
Osmolality	270-300 mmol/kg	↑ (Friend, 2000)
Cortisol	103-278 nmol/litre	↑ (Stull and Rodiek, 2000)

S= Serum, HP= Heparinised plasma, B= Blood

1.2.4.1. Assessment of food deprivation

Horses intended for slaughter in North America (Canada and USA) can be transported for more than 24 hours without food (Department of Agriculture, Animal and Plant Health Inspection Service, 2001; Department of Justice, 1990). Hence, food deprivation is a welfare issue which needs assessment in slaughter horses. Hunger is defined as an unpleasant feeling state caused by lack of food, coupled with the desire to eat (McMillan, 2003). Horses might not experience metabolic hunger as readily as other monogastric animal as there is a constant supply of energy for longer periods from the hindgut fermentation which in this respect makes them similar to ruminants. However, some studies have indicated that horses may be less capable of coping with hunger when compared to ruminants (Duncan *et al.*, 1990).

Wang *et al.*, 2006 describes the food deprivation physiology as follows: when monogastric animals are subjected to food deprivation, glucose becomes less available and hence triglycerides in adipose tissue are broken down to free fatty acids and glycerol. Food deprivation also produces protein breakdown in muscle and amino acids enter the circulation. When amino acids are deaminated, ammonia is released. Ammonia is potentially toxic and is converted into a safer form by the enzyme glutamate dehydrogenase to urea. Therefore, theoretically, blood glucose, free fatty acids, glutamate dehydrogenase and urea concentrations could all be used as indicators for evaluating energy mobilization. Friend, 2000 suggested that total bilirubin concentration can be used to assess starvation. When blood glucose is used as an indicator to assess food deprivation, the effect of glucocorticoids and other related hormones on the energy metabolism needs to be considered.

In one study, horses transported for a short distance (1 hour) showed a significant increase in blood glucose concentration (Werner and Gallo, 2008). It is obvious, that in such a short duration of transport, an effect of food deprivation on blood glucose will be negligible. Therefore, this increase in blood glucose could be attributed to a stress response due to release of adrenaline or epinephrine and cortisol (Gordon *et al.*, 2007). Horses transported for long distances (24 hours) with or without feeding provisions can also show an increased concentration of glucose (Stull and Rodiek, 2002) and cortisol concentration in blood (Friend, 2000). It is reasonable to assume from available scientific literature that increases in blood or serum glucose concentration can occur as part of a stress response (Gordon *et al.*, 2007) and may not necessarily provide useful information about hunger in horses during transport.

An increase in glucose concentration in horses has also been associated with respiratory disease during transport (Oikawa *et al.*, 2004). Blood glucose concentration can be elevated when horses are subjected to an additional stress such as cross tying during transport. When horses transported cross-tied were compared to loosely transported animals, the former had significantly higher blood glucose concentration than the latter (Stull and Rodiek, 2002). Glucose concentration showed peak elevation in each treatment group at the termination of transport. Also the return of glucose to pre-transit concentrations occurred more slowly than cortisol, with the loose treatment reaching pre-transit levels earlier than the cross-tied horses. The cross-tied treatment did not return to baseline until after 48 hours of recovery. These studies show that elevation of blood glucose concentration in response to stressful events is closely associated with the stress hormone which in turn causes energy mobilization.

Hypoglycaemia has been reported when horses are made to perform long periods of exercise. When horses were exercised in a steady trot or canter for 3.2 hours to 4.8 hours, their blood glucose concentration increased significantly when compared to basal concentration during the first half of the exercise regimen and dropped below reference range in the second half of the exercise regimen (Snow *et al.*, 1982). A study by Forhead *et al.*, (1995) also showed that when donkeys were subjected to fasting, by the end of the third day of fasting, glucose and insulin had decreased significantly in the fasted donkeys when compared to donkeys which are fed. This finding that blood glucose concentration decreases in response to fasting indicates that measuring blood glucose concentrations could be a useful indicator to assess welfare when transport durations are long and possibly when food deprivation is also an accompanying factor.

1.2.4.2. Assessment of thirst

Thirst is a feeling that is subjective whereas dehydration can be quantified indirectly by the reduction in body weight that can occur after an event such as transport (Friend, 2000). Dehydration has been defined as, “a disturbed state of hydration following water loss” (Mansmann and Woodie, 1995). Body weight reduction during the transport of horses occurs due to fluid loss from gut, respiration, sweat and reduced water intake.(Smith *et al*., 1996a; Berg *et al*, 1988).The magnitude of each type of fluid loss (extracellular and intracellular) can lead to different levels of dehydration. Some variables used in previous studies to identify and assess dehydration in horses are serum and plasma osmolality (Friend, 2000; Mejdell *et al.*, 2005) electrolyte concentrations (Butudom *et al.*, 2003; Friend, 2000; Iacono *et al.*, 2007) total plasma protein concentration (Friend, 2000; Iacono *et al.*, 2007) and packed cell volume (PCV) (Stull and Rodiek, 2002). Carlson *et al.*, (1979) deprived horses of food and water for 72 h at raised air temperatures and found significant increases in plasma osmolality and total plasma protein concentration, but PCV was not significantly increased.

Serum or plasma osmolality is an indication of the concentration of solute in serum or plasma and depends on the amount of solute (glucose, electrolytes etc) and solvent (water). Osmolality is one of the most closely regulated body variables in terms of homeostasis and hence could be an effective physiological variable to assess dehydration (Brownlow and Hutchins, 1982). Osmolality can be elevated or below from normal clinical range (hypertonic or hypotonic serum or plasma). A free water deficit causes a hypertonic dehydration, whereas in hypotonic dehydration, there is a relative greater loss of electrolytes than water (Engelking, 2004). Profuse sweating accompanied by immediate copious intake of water has been associated with hypotonic dehydration.

Transport of slaughter horses for long distances without feed and water could cause a hypertonic state and hence the measurement of plasma or serum osmolality can provide a good estimate of the level of dehydration. Brownlow and Hutchins, (1982) studied the plasma osmolality of 100 healthy horses and reported that the mean value was 282 (±6) mmol/kg. In hypertonic dehydration (true dehydration i.e. desiccation, associated with water deprivation), osmolality is greater than 300 mmol/kg (Brownlow and Hutchins, 1982).

Plasma total protein concentration and PCV are the other physiological variables often used as indicators of dehydration in horses. Stull and Rodiek (2002), Friend (2000) and Friend *et al*., (1998) found that plasma total protein concentration and PCV showed a significant increase associated with transport; however, they were within the normal clinical range both before and after transport and declined to baseline levels during the post-transport period. Peak levels of both measurements occurred during the last 12 hours of transport but were within the reference range for a normal horse (Stull and Rodiek, 2002). The increase in PCV during transport could also be due to splenic contraction from sympathetic stimulation rather than due to dehydration (Pösö *et al.*, 2004).

1.2.4.3. Assessment of fatigue

Fatigue is defined as "difficulty in the initiation of or sustaining voluntary activities" (Chaudhuri and Behan, 2004) and is thought to be accompanied by deterioration in performance (Jin *et al.*, 2009). The mechanisms of fatigue in events of different intensity and duration of exertion have been subject to much research, but no single cause of fatigue has been identified (Merck & Co., 2008). Some of the hypotheses suggested are lactic acid accumulation, muscle oedema, increased free radicals production, microcirculation changes and alteration in intercellular calcium exchange.

Horses on long journeys where they have to adjust their posture, continuously, in response to other horses and vehicular motion could become fatigued (Friend 2000). Physiological variables used previously to study fatigue in horses are blood lactate concentration (Stull and Rodiek, 2000; Stull and Rodiek, 2002; Werner and Gallo, 2008) and serum creatine kinase activity (CK) (Stull and Rodiek, 2000). These authors measured blood lactate concentration and creatine kinase activity along with behavioural variables to identify fatigue and concluded that fatigue is a common finding in horses after long distance transport. The plausible reason for fatigue during transport could be due to the efforts required to maintain balance during acceleration and deceleration in a moving trailer (Clark *et al.*, 1993; Waran *et al.*, 1996). Rational thinking suggests that road transport of horses could imitate aerobic exercise (slow and sustained exercise) which could be less dramatic than the anaerobic exercise model. However, fear inducing events during transport such as bad handling and bad driving (Woods and Grandin, 2008) may create fatigue through anaerobic exercise due to the severity of the muscular exercise.

Long-duration aerobic exercise can eventually lead to anaerobic exercise and cause an increase in plasma lactate concentrations (Worth, 1985). Hence, the concentration of lactate in blood, plasma or serum can be used as a clinical parameter to evaluate fatigue. Blood lactate concentration also provides information about the fitness of the horses sampled (Lindner *et al.*, 1992) because when horses are healthy, blood lactate concentration returns to basal levels within an hour after mild to moderate exercise. It is recommended to measure whole blood lactate concentration quickly after sampling rather than centrifuging the sample and measuring the serum or plasma lactate concentration because the red blood cells produce lactate after blood is drawn, causing a falsely elevated lactate concentration. An electrode analyzer with an enzymatic sensor can measure lactate concentrations in whole blood and overcomes the limitations of conventional enzymatic assays that are time-consuming and technically complex (Shimojo *et al.*, 1993). Stull and Rodiek (2002) showed that the serum lactate concentration did not increase significantly during 24 hours of transport.

Another physiological variable which has been used to understand muscle fatigue is creatine kinase (CK) activity (Sahlin *et al*., 2002). The enzyme creatine kinase is present in muscle, where it makes adenosine triphosphate available for use in muscle contraction by the phosphorylation of adenosine diphosphate from creatine phosphate. It appears in circulating plasma when there is tissue damage, particularly heart muscle or skeletal muscle tissue damage (Baird *et al.*, 2012). CK can be a very organ specific biomarker for muscles and can be differentiated between heart and skeletal muscle based on which isoenzyme activity is involved. Identification of the different creatine kinase isoenzymes present in the blood is a useful way of determining where damage has occurred even though some literature disputes this view point (Baird *et al*., 2012).

The usefulness of CK as an indicator for muscle fatigue in previous transport studies is inconclusive. For example, Friend, (2000) found that even after 30 hours of transport, the extreme muscle fatigue reflected by release of CK, as occurs during prolonged strenuous exercise (Snow *et al*., 1982) did not occur. CK was also not useful in differentiating between control horses, which had not been transported and loose horses that had been transported for 24 hours (Friend *et al.*, 1998). However, some other studies have interpreted a change in CK after transport when compared to basal concentrations as an indicator for fatigue (Padalino *et al.*, 2012; Tateo *et al.*, 2012).

1.2.4.4. Assessment of distress

Distress during transport and slaughter may be due to an emotional event such as fear and social disturbance. Fear in horses particularly during transport could be reduced by habituation and training (Weeks *et al.*, 2011). Fear involves physiological and behavioural changes that prepare the animal to cope with danger (Forkman *et al.*, 2007). Fear-related reactions can lead to injuries in horses due to the fight-flight mechanism accompanied by the release of adrenaline or epinephrine. For example, when in distress due to fear, a heifer can behave violently and can hurt itself (Boissy and Bouissou, 1995).

Cortisol concentration has been used by researchers to assess welfare during the transport of horses (Padalino *et al.*, 2012; Schmidt *et al.*, 2010; Tateo *et al.*, 2012). Plasma cortisol concentrations can increase as a result of fear and anxiety during transport (Fazio *et al.*, 2008) and hence could potentially be a useful indicator for stress assessment. Cortisol is secreted in response to activation of the hypothalamic-pituitary-adrenal axis during stressful situations. This may impact broad-based biological functions such as increasing blood glucose concentration for energy availability, suppressing immune competence and impairing reproductive functions (Stull and Rodiek, 2002). However, cortisol levels are highly influenced by individual variations (Flisinska-Bojanowska *et al.*, 1974), seasonal variations (Place *et al.*, 2010), gender, pregnancy (Flisińska-Bojanowska *et al.*, 1991) and circadian rhythm variations. In order to control for these variations, basal concentrations of cortisol in the horses transported or slaughtered should be measured and then comparisons should be made to the post stress values of those same horses.

Stull and Rodiek (2002) showed that cortisol concentration dramatically increased with loading and the initial phase of transport. When two treatment groups of horses, one group cross tied and another transported loose were compared, the cortisol concentration of the cross-tied group rose to a peak concentration at the termination of transport, while the peak concentration in the loose group occurred 3 hours into the journey (Stull and Rodiek, 2002). The cross-tied group had the highest peak concentration. Basal concentrations following transit were reached by the loose group earlier than in the cross-tied group. The sharp decrease of cortisol following unloading in both groups could be due to the elimination of the stressor (transport).

Short stressful events such as handling stress, isolation stress, and short-duration transportation are usually followed by an increase in the stress hormone cortisol. However, chronic stressful events such as long-acting heat stress or restraint for longer periods might not affect the basal hormone concentration or sometimes the basal concentration can be even lower. This could be due to down-regulation of corticotrophin-releasing hormone in response to high cortisol induced negative central nervous system feedback on further release of stimulating hormones (Guilliams and Edwards, 2010). An increase in cortisol concentration during long-term stress may lead to an increase in the neutrophil: lymphocyte (N: L) ratio (Stull *et al.*, 2008). Stull *et al*. (2008) suggests that N: L ratio is a more reliable indicator of chronic stress than cortisol concentration. Stull and Rodiek (2002) also found that there was a more substantial increase in cortisol and N: L ratio during transport for the cross-tied treatment than in the loosely transported horses.

1.2.5. Preference test and motivational test

Animal welfare science involves assessment of subjective feelings of animals (Duncan, 2005; Webster *et al.*, 2004). Hence, preference and motivational tests are important tools for assessment of welfare. Preference tests are commonly used to investigate 'what the animal prefers' given a particular situation. For example, when a preference test for food, exercise, paddock time alone and paddock time in groups were studied in horses, horses preferred food, paddock time and avoided forced exercise (Lee *et al.*, 2011). A similar preference test established that horses prefer highly grown pastures to improve their efficiency of grazing (Edouard *et al.*, 2009) than low pastures. The underlining principle for preference test is that animal is allowed to choose between certain types of feed or environment, and the assumption by researchers is that the animal will choose a feed or environment which will make the animal feel good and be in its best interest.

The problem with the preference test is that the animal's preference may be affected by previous experience, and in fact; the animal can choose the 'wrong' option, which can be bad for welfare and at times its own survival (Broom, 1991). A horse may choose to eat a lot of grains, which makes it feel good, but may expose him to laminitis and colic in the future. Another potential difficulty with a preference test is that there may be a conflict between short-term and long-term welfare. Horses which get no exercise (in maintenance diet) would prefer energy-rich food (grains) more than roughage when there is a choice provided to them leading to an overweight problem in the long term even though it may be harmless in the short term. One example of preference test used in the transport of horses is to identify the preferred orientation of horses during transport (Gibbs and Friend, 1999; Knowles *et al.*, 2010).

Motivational tests are useful to understand ‘how hard an animal will work to obtain food, water or a particular environment (Duncan, 2005). This is a method of asking the animal “what price it is willing to pay” for an event chosen, which may be a drinking or eating behaviour. In the case of slaughter horses, which travel long distances without food and water, measuring the strength of motivation can give us more understanding about the feelings of the horses on issues such as hunger, thirst and fatigue. For example, as most slaughter horses travel for long hours without feed and water, when unloaded at the lairage in a slaughterplant they might lie down to rest rather than eat and drink even though they are hungry and thirsty, which could indicate that the feeling of fatigue was stronger than that of thirst and hunger.

1.3. WELFARE ISSUES ASSOCIATED WITH THE TRANSPORT OF HORSES BY ROAD

Horses have been transported by road in large numbers since the beginning of the 19th century for war-related activities (Cregier, 1982) and currently for sporting reasons, draught activities, pleasure activities and also for slaughter (Waran *et al.*, 2002). Transport of horses is an essential part of any equine production system (Leadon, 1994). Horses used for sport are likely to be transported more frequently than other types of livestock such as sheep or pigs, which may only be transported for slaughter and management procedures such as fattening. Friend (2001) after conducting field-based studies on horse transport opined that horses generally adapt well to transport if the conditions are optimal. Efficient and proper care during transportation can reduce the likelihood of ill effects on the health and welfare of horses.

A considerable amount of scientific work has already been performed for some of the welfare issues associated with the transport of horses such as injury (Grandin *et al.*, 1999) and disease (mainly respiratory) (Oikawa *et al.*, 2004; Stull and Rodiek, 2002). However, for other welfare issues such as thirst (Friend, 2000), fatigue (Friend, 2000; Mansmann and Woodie, 1995), fear, pain and hunger more research is needed for identification and quantification (Cockram *et al*., 2012). In long distance transport, it is difficult to demonstrate or quantify the magnitude of the welfare problem as they are subjective feelings. For example, Friend (2000) observed "obvious" signs of fatigue in horses that had been transported for 30 h without water, in hot and humid conditions. The signs of fatigue identified, but not quantified, were an absence of social interactions, a reduced response to stimuli and a "depressed" appearance.

Risk factors for reduced health and welfare for horses being transported may be resource-based (e.g. vehicle type, stocking density) animal-based (e.g. fitness, biting, kicking, fear, fatigue) or environment based (e.g. temperature and humidity inside the vehicle). Resource-based factors which have been identified to affect welfare are poor air quality in the vehicle (Leadon *et al.*, 1989), improper loading and unloading conditions (Waran and Cuddeford, 1995), vibrations of the vehicle (Smith *et al.*, 1996b), stocking density (Collins *et al.*, 2000; Knowles *et al.*, 2010) and handling methods used while loading and unloading. Providing even basic resources such as feed, water and rest for horses during long distance road transport can increase costs in commercial transport of horses for slaughter but if these resources are not provided, then there is the potential for hunger, thirst and discomfort to occur. Animal based factors, which are identified to affect welfare are fitness before transport (Grandin *et al.* 1999), kicking and biting behaviour leading to injuries (Grandin *et al.* 1999), fear and fatigue (Giovagnoli *et al.*, 2002).

Factors which can affect the welfare of horses during transport are discussed in detail below in no specific order of importance.

1.3.1. Fitness before transport

Fitness of the animal before transport (i.e. soundness or freedom from illness and injury) is an important factor that can affect the welfare of horses. Grandin *et al.* (1999) reported that eight percent of horses transported for slaughter had severe welfare problems that would have made them unfit for travel. After observing 161,685 horse movements through the road and railway border stations in Sezana and Port of Koper in Slovenia, Marlin *et al*. (2011) reported that one of the reasons for a high injury rate among slaughter horses was that unfit horses were transported. These studies underline the importance of fitness of horses before transport. Some horses, even though they are physiologically fit, may need special husbandry provisions for transport. This category of horses includes large or obese horses, excitable or aggressive horses, untrained horses and horses which have certain injuries (OIE, 2012). The OIE recommends that it is important that an appropriate authority or the animal owner himself assesses the animal for fitness before transport. The inspector or animal owner should look for horses that are sick, injured, weak, disabled or fatigued, unable to stand unaided, bear weight on each leg, blind, newborn, pregnant (particularly in the third trimester of gestation) and in poor body condition, and make a decision not to transport them (OIE, 2012).

1.3.2. Loading and unloading

Loading and unloading of horses for transport is a critical point when injuries or a stressful event can occur (Shanahan, 2003). Apart from becoming injured during loading and unloading, physiological and behaviour variables have also shown indication of stress response of horses (Waran and Cuddeford, 1995). In the study by Waran and Cuddeford (1995) increased heart rate was recorded in all age groups of horses and aversive or evasive behaviours were observed more often with inexperienced horses than experienced horses. Plasma lactate concentration was been shown to increase in horses immediately after loading when compared to one hour prior to loading (Werner and Gallo, 2008). This could be due to high muscle activity. Increased heart rate was also recorded during loading and unloading when compared to pre transport and during transport (Waran *et al.*, 1996). Some literature suggests that horses are reluctant to enter a dark area while loading (Waran *et al.*, 2002). However, other literature suggests that there is no difference in the behaviour of horses due to lighting while loading of horses on to a trailer (Cross *et al.*, 2008).

The above mentioned studies provide empirical evidence that loading and unloading is a stressful event and hence proper management such as good handling techniques and good design of the loading and unloading ramps are necessary to reduce the impact. Guidelines developed by Department of Environment, Food and Rural Affairs, UK (DEFRA) suggest that ramps used for loading and unloading should not have a slope exceeding 20 degrees (DEFRA, 2007); however scientific studies on details regarding the effect of slope of the ramp for ease of loading of horses are lacking. DEFRA also recommends a raised unloading bay as a basic requirement for slaughter plants, which deal with horses, as it helps them to walk out of the vehicle without slipping. Loading can be a fearful procedure for horses if carried out by inexperienced or insensitive personnel.

In the case of horses that have been transported for slaughter, they need to be unloaded as soon as possible in to the lairage as the distances travelled can be long. Once a vehicle arrives at a slaughterplant it is in the animals' best interest that they are unloaded immediately. On a fully loaded vehicle, even in cold conditions, there will be a build-up of heat and humidity that may compromise their wellbeing (Farm Animal Welfare Council, 2003). A well designed, constructed and maintained unloading area can add to the speed and efficiency of unloading, consequently limiting the amount of stress on animals. The unloading area should not have any sharp turns. Covered unloading areas allow lighting levels to be set to encourage animals to come off the vehicle. Unloading areas with solid sides rather than open rails facilitate unloading and prevent animals from being distracted or alarmed by external movements (Farm Animal Welfare Council, 2003).

1.3.3. Feeding and watering before, during and after transport

To provide optimal welfare standards to horses which are being transported, it is important to understand the feeding and drinking pattern of horses during transport. There is evidence to indicate that the drinking behaviour of a horse may be affected by transport. When water intake was measured in horses transported for 24 hours, during which water was provided at rest periods, and comparison was made to that of stabled horses with ad-lib water, water intake was significantly reduced during transport (Smith *et al.*, 1996a). Berg *et al.* (1998) showed that thoroughbred horses drank less in the first 8 hours of transport than control horses which were not transported. The transported horses were three % dehydrated when the vehicle temperature was between 19.4 and 27.9º C. This study also found that feed consumption was not affected during transport when compared to control horses, which were not transported (Berg *et al.*, 1998). However, another study showed horses tend to eat less while in a moving vehicle compared to when the vehicle is stationary (Kusunose and Torikai, 1996). Generally, it is considered good husbandry practice to provide water before the start of transport. The Canadian Health of Animals Regulation and the USDA's Slaughter horse transport program recommend the provision of water before the journey starts (Canada Department of Justice, 1990; Department of Agriculture, Animal, and Plant health inspection services, 2001)

According to European Council legislation, the feeding and watering of horses during transport gains significant importance in terms of welfare, when the duration of travel increases beyond 8 hours. Journey duration and stocking density are two factors which are clearly and objectively defined in the European Council legislation (Gavinelli and Simonin, 2003) to protect horses from suffering during transport. Transporting healthy horses for 24 hours, even with provision of food during transport using hay nets and water being provided before transport and during rest periods (every 3.75 hours for 0.25 hours), can induce slight dehydration (Smith *et al.*, 1996a). Twenty-four hours of transport without food and water produced dehydration level of eight percent of body weight, whereas non-transported horses penned without food and water for 24 hours developed a dehydration of 10 percent (Friend, 2000). Even though, horses are capable of tolerating up to 12% dehydration in terms of body mass (Sneddon *et al.*, 1991), health and welfare consequence could be severe. Analysing this scientific evidence indicates that horses can travel for at least 10 to 12 hours without feed and water without showing obvious signs of dehydration, however this is conditional on the provision of feed and water before the start of transport and provided that the environmental conditions are optimal inside the vehicle.

As discussed earlier, horses tend to drink less during transport and hence adequate provision of water should be made at the end of the journey for them to compensate. Friend (2001) showed that four hours after transport was completed, and the horses had received two offerings of water, most indicators of dehydration such as plasma osmolality, and sodium and chloride concentrations returned to normal (Friend, 2001). The scientific panel of the European Commission recommends that horses should have continual access to an unrestricted supply of clean drinking water for a period of one hour before transport and for one hour immediately following transport (EFSA Panel on Animal Health and Welfare, 2011).

1.3.4. Rest periods

Research work on the usefulness of rest periods during transport of horses has been inconclusive. There are two types of rest-stops discussed during the commercial transport of horses. First, rest-stops can be done with the horses resting for a certain period inside the vehicle itself while the vehicle is not moving and second, horses can be unloaded from the vehicle for rest. Some studies have shown that rest periods can be used effectively to reduce the impact of transport stress on horses. For example, Calabrese and Friend (2009) determined that slaughter horses transported in groups, when given a one hour rest period inside the vehicle itself, tend to show reduced frequency of movements compared with that of during transport. Mean movement rate tended to be higher during travel (4.23 m/hour) than during the 1-hour periods when the truck was stopped (2.75 m/hour). However, movement of horses inside the vehicle can be affected by stocking density and duration of transport. It is possible that the reduced movement of horses during these rest periods could provide some rest due to the absence of vehicular movements, hence can have positive effect on welfare. There are other studies, which argue that rest periods outside the vehicle do not effectively reduce stress levels because unloading and reloading horses to provide rest can be stressful.

The inclusion of a 12-hour rest-stop by unloading the horses half way into a 24-hour journey (transported under commercial conditions) did not have any effect on the serum cortisol concentration, total blood leukocyte, neutrophil and lymphocyte counts, when compared to horses, which were transported without rest stop (Stull *et al.*, 2008). However, the 12 hour rest-stop interrupted the transport-related decline in the blood lymphocyte subpopulations and allowed them to recover towards their resting levels. During a one-hour on-truck rest period after 8 hours and 16 hours of transport, the activity of horses was similar during the first and second rest stops, indicating that these horses were not fatigued to the point of reducing activity after 16 hours of transport (Friend *et al.*, 2006). Another study reported that increasing the rest time (horses rested inside the vehicle) and cleaning the interior of the vehicle during rest stops reduced transportation stress and microbial infection of the respiratory system (Oikawa *et al.*, 2005).

1.3.5. Vehicle type

The common types of vehicles used in the USA and Canada (Grandin *et al*., 1998; Stull, 2008) for the transport of horses are:

- Ordinary vehicle converted to transport horses,
- Livestock trailer attached with a hitch to draw/tow-bar,
- Horse trailer (livestock trailer with internal partitions for horses),
- Gooseneck trailer attached to pick-up truck with 5^{th} wheel coupling,
- Single-deck articulated livestock vehicle (cab and trailer),
- Single-deck articulated horse vehicle (cab and trailer with internal partitions for horses)
- Multi-deck vehicle.

In the summer (July and August) of 1998, Grandin *et al*. (1998) observed 1008 horses in sixty-three trailer loads that arrived at two slaughter plants in Texas. Forty-two percent of the horses were transported in double deck trailers, nine percent in straight single-deck semi-trailers and 49% in gooseneck trailers. The use of multi deck semi trailers (potbelly) with a capacity of 44 horses used for the transport of slaughter horses was stopped in the USA following regulation in December, 2006 (Department of Agriculture, Animal and Plant Health Inspection Service, 2008). Stull (1999) and Grandin *et al*. (1998) reported that the percentage of injured horses was greater for two-tiered "pot-belly" compared to single-deck trailers. They argued that the double-deck trailers are primarily designed to transport cattle. Cattle generally require less head room than horses and hence multi-deck trailers may not be suitable to transport horses.

Stress indicators such as serum cortisol concentration, neutrophil: lymphocyte ratio and rectal temperature showed significantly greater responses following transport in single-deck trailers when compared to double deck trailers (Stull, 1999). But others contested this claim by arguing that this particular finding about stress indicators may have been due to poor ventilation of the straight deck vehicle used in this study (Houpt, 2007) and had nothing to do with the comparative lower stress in a multi-deck vehicle. The increase in prevalence of injuries in a multi-deck vehicle can merit an intervention to stop the usage of multi-deck vehicles for transport of horses.

The Terrestrial Animal Health Code of OIE provides detailed guidelines on many aspects of vehicle design, for animals transported for slaughter (OIE, 2010). Some of the salient aspects of this regulation are:

- Vehicles should be designed appropriately for the size of the animal and should have no protrusions, which can injure them.
- Should provide protection against inclement weather.
- Should be easy for cleaning and disinfection.
- Should have adequate ventilation.
- Should have good flooring, which is not slippery and soft enough not to cause injury to the animals if the animal falls down.
- Should have proper side padding, partitions, roof, and lighting.

1.3.6. Vibration and acceleration

The vibration and acceleration of the vehicle during transport can have an impact on the welfare of farm animals by affecting the postural stability, functioning of internal organs and muscle fatigue (Gebresenbet *et al.*, 2011). When pigs were subjected to vertical vibrations, stress hormones (adrenocorticotrophic hormone and cortisol concentrations) showed significant elevation, in comparison to basal concentration before vibration (Perremans *et al.*, 2001). Similar results were replicated in calves in terms of elevation in cortisol concentration (Van De Water *et al.*, 2003). Vibrations during transport could be multi-dimensional (vertical, horizontally and possibly more) which might be difficult to recreate in an experimental setting. Sheep showed significant elevation of cortisol concentration when driven on rough secondary roads in comparison to smooth highways (Monge *et al*., 2009; Ruiz-de-la-Torre *et al*., 2001). The above-mentioned studies provide evidence that transport vehicle vibration is an important factor which needs to be controlled during the transportation of horses.

There have been limited studies to evaluate the effect of vibrations and acceleration on horses in terms of welfare during transport. In one study in USA (where traffic flows on the right side of the road) horses loaded on the right side of the trailer experienced more vibration than horses on the left side of the trailer, perhaps because of the poor conditions of asphalt on the edges of the roads (Smith *et al.*, 1996b). Smith *et al*. (1996b) also evaluated the effect of types of suspension tire, inflation rate and presence of shock absorber for smoothness of the ride during the transport of horses. The leaf spring suspension with low pressure radial tires and without shock absorbers provided the smoothest ride for horses as measured by accelerations (m/s). In another study, when the movement of the vehicle was increased experimentally by abrupt stops (irregular acceleration and deceleration), horses tended to show less adaptation in terms of behaviour adjustments (but they showed a difference in orientation when compared to horses transported with stable acceleration) (Kusunose and Torikai, 1996). The information from these studies suggests that a well-designed vehicle to reduce vibration, good roads and trained drivers who drive smoothly are important aspects to reduce welfare concerns during transport.

1.3.7. Environmental conditions

The thermal environment prevailing in the vehicle could potentially be a factor affecting welfare, particularly for horses destined for slaughter. This is a consideration particularly because these horses are subjected to the commercial standards of transportation of farm animals, which are different from transport of sport or companion horses. Horses may start to exhibit a thermal stress response when the temperature inside the vehicle goes beyond or below the range of upper and lower critical temperature for horses (Stull, 1997). The upper critical temperature for horses has been estimated to be 25º C to 30ºC (Honstein and Monty, 1977). The lower critical temperature however could be as low as -15ºC for horses (McBride *et al.*, 1985). Thermal stress as measured by an increase in respiration rate and skin temperature has been documented in ponies exposed to ambient temperatures above 30°C (Kaminski *et al.*, 1985).

Apart from the environmental temperature, there are many factors which can have an effect on the internal micro-environment of a vehicle loaded with horses. Stocking density, ventilation design and humidity inside the trailer can have an impact on the micro-environment inside the vehicle (Purswell *et al.*, 2010). The ventilation should be optimum to maintain air quality inside the vehicle similar to that of outside the vehicle. However, during winter when the temperature can reach -30 º C in Canada, excess ventilation for air quality can create thermal stress due to cold draft. Purswell *et al.* (2010) demonstrated that temperature differences between inside and outside the vehicle decreased with increasing vehicle speed and open vent area and increased with the number of horses present. This study also suggested that heat stress conditions were likely to occur in horse trailers with the current ventilation systems and suggested improvements in trailer design to increase ventilation (Purswell *et al.*, 2010). Stull and Rodiek (2002) suggested that when the relative humidity inside the vehicle was higher than 50%, that affected the ability of horses to dissipate heat and hence made them more susceptible to heat stress.

Purswell *et al*. 2010 suggested that the thermal comfort of horses during transport could be assessed effectively using tools such as the International Equestrian Federation (FEI) comfort index or the Wet Bulb Globe Temperature (WBGT). The FEI's comfort index adds up the relative humidity percentage and ambient temperature in Fahrenheit. WBGT takes into account the conductive, convective, radiation and evaporation loss of temperature and could be estimated using the formulae:

$$WBGT=0.7T_{wb}+0.3T_{g,}$$

where T_{wb} is wet bulb temperature and T_g is the black globe temperature (Schroter and Marlin, 1995).

1.3.8. Stocking density

Stocking density and space allowance are two terms generally used by the scientific community to quantify the space provided for animals during transport. Quantifying the space provided for animals will help us mitigate welfare issues associated with overcrowding. Generally, stocking density is expressed as kg/m^2 and space allowance is expressed as m^2/animal. Field studies in the USA have reported that horses used for recreation and sport are generally given a space allowance of 1.4-2 m^2/horse and commercial trailers generally provide a space allowance of 1.1 to 1.5 m^2/ horse (Stull, 2008). The commercial trailers studied by Stull (2008) had an average number of 28 horses on each load of double deck trailers, 22 horses on single-deck straight trailers and 11 horses on gooseneck trailers.

The Canadian Code of Practice recommends a stocking density of 400 kg/m^2 for an adult horse weighing 450 kg and with good body condition (NFACC, 1998). When this recommendation is converted to space allowance, it is equivalent to 1.1 m^2 for a horse weighing 450 kg. Stull (2008) reported that commercial transporters of horses in the USA provided 1.1 m^2 space allowances during transport. If the Canadian and the USA transporters provide the same space allowance currently, then they are in compliance with the Canadian Code of Practice. Estimation of stocking density in Chapter 6 indicated that similar space allowance was provided for horses transported for slaughter currently. However, if a horse weighs more than 450 kg they needs to be provided with more space allowance according to the increase in weight.

The Canadian Code of Practice recommendations regarding stocking density were derived from a study by Whiting (1999). This study estimated the stocking density by observing horses destined for slaughter in Canada and suggested that the recommended loading density for horses transported in groups as $y = (54.837)\ W^{0.325}$, where y = density in kg/m^2 and W = average animal weight in kilo grams. This formula, converted into the space allowance per animal, is approximately: $A = (0.0182)\ W^{0.675}$, where A is the space allowance in m^2 per horse and W is the average weight of the horse in kilograms (Whiting, 1999).

When horses were transported with space allowances of 1.28 m^2 /horse and 2.23 m^2 /horse, the group of horses provided with low space allowance showed a significantly higher incidence of falling and injury than horses provided with high space allowance (Collins *et al.*, 2000). Collins *et al.* (2000) used slaughter type horses in this study and used poor driving quality (hard breaking and rapid accelerations) for 25 minutes as a stressor. Knowles *et al.* (2010) also found that there was a reduction in the number of slips, falls, stumbles and collisions during transport, when the space allowance provided was high. However, Stull (1999) reported that the percentage of horses injured was lower in trailers with 1.14 to 1.31m^2 of floor area per horse than in trailers with 1.40 to 1.54 m^2 of floor area per horse. Knowles *et al.* (2010) studied untamed feral ponies transported in groups and found a positive association between aggressive behaviours and stocking density whereas Iacono *et al.* (2007) studied slaughter horses in USA and did not find any association.

1.3.9. Headroom or deck height

Head injuries when horses intended for slaughter are transported under commercial conditions have been widely reported (Grandin *et al.*, 1999; Stull, 1999) and lack of adequate head room or deck height has been implicated for these injuries. Multi-deck trailers have a high prevalence of head injuries compared to single-deck trailer, which have more headroom (Stull, 1999). This provides evidence that head room is a factor associated with head injuries. However, a considerable percentage of horses transported in single-deck trailers also had head injuries. Therefore, there is a need to investigate all factors associated with head injury and the magnitude of the association between head room or deck height and the incidence of injuries.

The Health of Animals Act and Regulation, Section 142 states that, “no person shall transport animals that are unable to stand in its natural position without coming into contact with a deck or roof” (Department of Justice, 1990). The Canadian Code of Practice for equines goes further by describing ‘natural position’ as having full unobstructed range of head and neck motion (NFACC 1998). It also suggests that provision of 2.5 cm of head space for every 10 cm of height of the horse at the withers could be used as a guideline. An investigation focusing on the headroom for horses transported came to the conclusion that 2.5 cm of head space for every 10 cm of height at the withers may not be sufficient to avoid injuries (Whiting and Sauder, 2000). United States Animal and Plant Health Inspection services also investigated this problem and decided to stop the usage of multi-deck transport trailers for slaughter horse transport, which generally provided less head room than the single-deck trailers (Department of Agriculture, Animal and Plant Health Inspection Service, 2001).

1.3.10. Injuries

The incidence of wounds, fractures and bruising are significant welfare issues reported in slaughter horses during transport (Grandin *et al.*, 1999; Marlin *et al.*, 2011). Grandin *et al*. (1999) observed 63 truckloads containing 1008 horses in two slaughter plants in USA and reported a prevalence of two percent severe wounds and 25% bruising in the carcasses. Stull (1999) after studying nine trailer loads of horses transported for slaughter, reported 20% of horses sustaining wounds (single or multiple abrasions and lacerations). The high amount of variability regarding prevalence of wounds between both studies (2 and 20%) could be due to differential classification of wound categories. The former study only took into account severe wounds, such as deep cut wounds, whereas the latter also accounted for even small wounds.

Factors associated with prevalence of wounds and other physical injuries are vehicle design, stocking density, aggression between horses, sex, journey duration and fitness of horses transported. Grandin *et al*. (1999) reported that in 50% of the horses which had bruises, the bruising was attributed to kicks and bites, thereby indicating fighting behaviour or aggression during transportation of these animals as a major concern for welfare. This study also identified that cut wounds and lacerations increased with the number of rest stops during transport, which needs further investigation.

Stull (1999) reported that among the injured, most injuries (58%) were on the head and face. Head and injuries around eyes were also associated with transport on double deck trailers with less head space. More horses were injured in potbelly (29.2%) than straight-deck trailers (8.0%). The percentage of horses injured was higher for long trips (33%), compared with trips of short (8%) or medium duration (9%). Stull (1999) considered 27 to 30 hours as long duration transport, 16 to 23 hours as medium and less than 6 hours as short-duration transport.

Collins *et al*. (2000) studied the effect of space allowance on injuries and reported that the incidence of injury was significantly higher at low space allowance (64%, 1.28 m^2/horse with 14 horses) than at the higher space allowance (29%, 2.23 m^2/horse with eight horses). However, there was no significant difference in the average severity of injury between these two groups. After observing 8016 truckloads (262, 929 cattle and 161, 685 horses) in Sežana and Port of Koper, Slovenia, Stefancic and Martin (2005) reported a higher mortality rate due to injuries during the transport of horses than that of cattle. Marlin *et al.* (2011) observed horses transported for slaughter in Europe and also opined that horses may be more prone to injury than cattle.

1.3.11. Journey duration

Journey duration is an important factor which can affect the health and welfare of horses transported for slaughter under commercial conditions (Marlin *et al.*, 2011; Stefancic and Martin, 2005; Stull, 1999). When federally approved slaughter plants were operating in the USA, some horses intended for slaughter were transported for distances exceeding 2575 km, with travel times in excess of 30 hours (Reece *et al.*, 2000). Reece *et al*. (2000) also indicated that Canadian slaughter plants sent trucks on a pick-up circuit through the USA that involved travel as far as South Carolina, Georgia, and Alabama before returning to Canada with a load of horses for slaughter. The trend of Canadian slaughter plants transporting horses for long distances from the USA is continuing as illustrated in chapter 2. Reece *et al.* (2000) and other authors predicted this trend in 2000, and argued that, as the number of horses available for slaughter in the USA increased and the number of horse slaughter plants decreased, there would be an increased possibility of horses being transported over longer distances thereby increasing the duration of journey for slaughter.

Horses transported for slaughter are provided with fewer provisions (Iacono *et al.*, 2007) than pleasure horses and may have reduced space allowance, less access to feed and water and hence their welfare may be at a greater risk, particularly when the journey duration increases. Stull (1999) studied the effect of transport duration on horses intended for slaughter and reported that when journey duration increased from 6 hours to 30 hours, the magnitude of the difference between the pre and post transport values of serum lactate concentration, haematocrit and serum total protein concentration increased significantly. Similar results were obtained by Friend (2000) who studied the physiological variables of horses, which are transported up to 30 hours without access to water. However, behavioural observation in these slaughter horses has identified signs of fatigue (as measured by the behaviours: closing of eyes, lower head carriage, less social interaction and fewer responses to stimulation) after 24 h of transport. These findings suggest that horses should not be transported for longer than 24 hours if they do not have access to water during hot conditions and not transported for longer than 28 h, even if they have periodic access to water (Friend, 2000). Another important factor to consider regarding the duration of rest in long duration transport is the ability of the horses to sleep (Mellor *et al.*, 2009).

1.3.12. Orientation and restraint

Some studies have suggested that horses have a preference for rear facing orientation (away from the direction of traffic) when in transit. Waran *et al.* (1996) found that horses positioned facing the rear side of the vehicle had a lower heart rate than horses facing the direction of travel. Horses facing rear tended to rest more on the buttock region and showed fewer movements and vocalization than horses facing forward towards the direction of travel (Padalino *et al.*, 2012; Waran *et al.*, 1996). However, these results have often been contradicted because of differences in trailer design and individual preferences of horses. A similar study looking at the heart rate of rear and forward facing horses found no significant difference between the two groups (Smith *et al.*, 1994). Toscano and Friend (2001) studied the effect of orientation of horses on the ability of the horses to balance themselves during transport. This study also indicated that orientation may not be a significant factor in maintaining balance during transport of horses. Collins *et al*. (2000) indicated that horses did not have a natural preference for one direction or another. Friend (2001) reported that orientation either towards or away or diagonally from the direction of travel does not seem to affect the ability of a horse to maintain its balance. When transported loose, there is an indication that horses mostly prefer to face rearwards, though this has been disputed by other studies. Gibbs and Friend (1999) reported that loose horses when transported tend to face towards the direction of travel whereas Knowles *et al.* (2010) reported that they tend to face away (rear facing) from the direction of travel. In short, studies have not clearly identified any welfare benefit because of a particular orientation during transport.

Horses can be restrained during transport by tying individual horses (using head collar/halter and a rope) and also by different modes of segregation (e.g. dividers). Tying individual animals can cause restriction of head movement (particularly the ability to lower the head) which has been associated with increased respiratory infections in horses (Stull and Rodiek, 2002). As discussed in sections 1.2.4.1 & 1.2.4.4 about assessment for food deprivation and distress, elevation of blood glucose concentration and plasma cortisol concentration was also associated with restraint during transport.

1.3.13. Dehydration and weight loss

Friend (2000) studied the effects of transport on the hydration status of horses and reported that horses transported for 30 hours without water show >10% dehydration. This study indicated that horses showed marked dehydration after 24 hours of transport and extreme dehydration after 28 hours of transport in hot and humid conditions (temperature ranged from 24 to 37ºC and percentage relative humidity ranged from 32 to 94). The findings of this study corroborate the previous studies performed by Stull (1999) and Smith *et al*. (1996). Stull (1999) reported that when horses were not provided with any feed or water during transit, they showed a linear increase in plasma total protein concentration with journey duration. Smith *et al*. (1996) reported that when horses were transported for 24 hours with provision of feed and water in-between transport (feeding provided by hay nets during transport and water provided during rest periods which was at every 3.5 hours intervals of transport); there was a significant elevation of packed cell volume and a non-significant elevation of plasma total protein concentration. This evidence suggests that horses should be provided with adequate provisions for drinking water, particularly during long distance transport of more than 20 hours.

Dehydration leads to cascading changes in physiology which in turns affects the health and welfare of the animals (Mansmann and Woodie, 1995). For example, dehydration affects the cardiac output and thereby affects thermoregulation in horses (Naylor *et al.*, 1993) making them prone to heat and cold stress. Naylor *et al.* (1993) suggested that when horses were dehydrated, there was decreased transmission of heat from the core of the body to the periphery, thereby affecting thermoregulation.

1.3.14. Fatigue

Fatigue has been reported in horses, which are transported for long distances, possibly due to the effort required by the animal to maintain balance during acceleration and deceleration in a moving trailer (Clark *et al.*, 1993; Giovagnoli *et al.*, 2002; Waran *et al.*, 1996). As discussed above in the "assessment of welfare "section (section: 1.2), most studies on fatigue used behavioural signs and physiological variables to assess fatigue. Factors which have been associated with fatigue during transport of horses are journey duration, lack of provision of food and water, vibration and restraint during transport. Friend *et al.* (2006) found that horses transported (in groups) for 16 hours with or without water, showed good activity levels and no behavioural signs of fatigue. This indicates that horses are able to cope without showing any behavioural signs of fatigue when transported for 16 hours. However, the same author reported that when horses were transported for 28 hours, they showed behavioural and physiological indicators of fatigue (Friend, 2000). Stull and Rodiek (2002) observed a significant elevation in serum lactate concentration when values before and after transport were compared. In this study, horses were transported for 24 hours without feed or water (Stull and Rodiek, 2002). This study also indicated that serum lactate concentration was also not affected by the freedom of movement for horses (restricted by cross tying or not restricted) (Stull and Rodiek, 2002). Serum creatine kinase activity did not increase above the normal clinical range even after long distance transport (30-hour journey duration) in one study (Friend, 2000). However, in another study, serum creatine kinase activity was higher in horses transported for 200 km when compared to horses transported for 50 km (Tateo *et al.*, 2012).

1.3.15. Health risks

Injuries, respiratory infection and colic are the commonly reported health risks associated with the transport of horses (Grandin *et al.*, 1999; Marlin *et al.*, 2011; Oikawa *et al.*, 2005; Raidal *et al.*, 1997b; Stull *et al.*, 2008). Grandin *et al.*, (1999) considered that 1.4% of horses at two slaughter plants in the USA were not fit to have been transported, 7.7% had pre-existing conditions or injuries present before loading, 3% were emaciated and 1% had conditions that affected mobility. Injuries during transport could be superficial bite wounds, severe wounds, bruising and fractures. Some of the risk factors for these injuries are slips and falls (Collins *et al.*, 2000), bites and kicks (Grandin *et al.*, 1998) and accidents. Respiratory infection has been reported as a major health risk in horses during and after transport. Smith *et al*. (1996) reported that there was no significant effect of transport on pulmonary aerosol clearance rates in horses transported for 24 hours under comfort zone conditions for ambient temperature and humidity. Another study reported that when horses were transported for 12 hours, the bacterial count and purulent secretions increased in the tracheal aspirates, however, all animals were able to clear the infection effectively after transport (Raidal *et al.*, 1997a). Japanese studies, which used closed vehicles for the transport of horses with provision of hay and water, have shown a high incidence of respiratory infection (Oikawa *et al*., 2005). Studies which analysed the relationship between colic and transport of horses did not show consistent association due to other interacting factors such as feeding and housing managemental changes (Archer and Proudman, 2006).

1.3.16. Confinement, group size and composition

Confinement in the vehicle during transport limits the range of normal behaviour of the animal and may affect welfare. In most cases, the transported horses cannot turn around or move freely (Leadon *et al.*, 2008). Confinement and isolation in stalls did not result in any significant change in adrenal function or cortisol concentration (Mal *et al.*, 1991). More investigation is needed on whether confinement and isolation during transport have any major effects on the welfare of horses. Isolation and confinement can be necessary during the transport of horses due to the increased risk of injuries from biting and kicking. According to the scientific panel which collected evidence for developing the European Union Animal Welfare Regulations (EFSA Panel on Animal Health and Welfare, 2011), the use of a partition should be compulsory. This panel suggests that horses may find it relatively difficult to maintain their posture during sudden vehicle movements and hence partitions between stalls should protect and physically isolate each animal. However, the absence in the regulations (European Commission, 2005, Regulation (EC) No 1/2005) of a specific definition of what constitutes a partition has been criticised as inappropriate designs can cause injury (World Horse Welfare, 2008). If horses are isolated during transport, provision of appropriate space allowance becomes essential so that they can rest comfortably while standing and will not get injured even if they lay down.

Group size is another factor, which can affect the welfare of horses when transported in groups. A small group size of four horses during transport has been reported to be less stressful than complete isolation for young animals and feral ponies (Knowles *et al.*, 2010). To avoid injuries, care should be taken in the allocation of the composition of groups while planning the journey. Even though, it is generally accepted that equines are social animals, there have been incidences when horses that are mixed together can exhibit aggressive behaviour such as biting, particularly when they are transported in groups (Iacono *et al.*, 2007). Mares travelling with foals should travel together with increased space allowance. Stallions should never be mixed with mares or geldings during transport.

1.3.17. Handlers, driver training and competence, and animal inspection

Training of staff involved in transport and handling and having guidelines regarding the training of staff can have a significant effect on the ways in which handlers manage animals (Broom, 2007). The behaviour of a driver towards animals during loading and unloading and the way in which he/she drives the vehicle are affected by the criteria for payment (Broom, 2007). Outcome based rewards for the transporter may help improve welfare. Regulations regarding training of handlers, drivers and inspectors vary in different countries. The Terrestrial Animal Health Code of the OIE recommends that the owner of the animal has to ensure the presence of an animal handler competent for the species being transported during the journey with the authority to take prompt action. In the case of transport by individual trucks, the truck driver may be the sole animal handler during the journey. Animal handlers are responsible for the humane handling and care of the animals, especially during loading and unloading, and for maintaining a journey log.

Managers of the facilities should ensure that loading and unloading ramps, access doors, handling equipment, halters and blindfolding equipment are all ready and functional. Managers are responsible for provision of water and feed when required, and protection from adverse weather conditions until further transport, sale or other use (including rearing or slaughter). The responsibilities of competent authorities other than the animal handler and driver as laid out by the OIE are:

- “Establishing minimum standards for animal welfare, including requirements for inspection of animals before, during and after their travel, defining ‘fitness to travel’ and appropriate certification and record keeping.
- Setting standards for facilities, containers and vehicles for the transport of animals.
- Setting standards for the competence of animal handlers, drivers and managers of facilities in relevant issues in animal welfare” (World Organization for Animal Health (OIE), 2010).

1.3.18. Transport-related regulations

International, national and provincial or state regulations provide protection to horses regarding health and welfare during transport. First, international organisations create regulations to prevent obvious types of abuse during transport. One example is the European Council Regulation (EC) No 1/2005 (The European Council, 2005) which is binding on all European Union member states. It has special regulations for horses intended for slaughter. Some of the regulations are on stocking density for various modes of transport for horses, feeding interval, journey duration and resting periods. According to these regulations, journey durations exceeding eight hours are considered long journey times and need special resource requirements such as decreased stocking densities, special vehicle requirements and rest periods (Gavinelli and Simonin, 2003). Another example is the World Organization for Animal Health (OIE) with 177 members; it is an international reference organization which elaborates broad guidelines for the transport of equines in the Terrestrial Animals Health code.

Second, there are minimum welfare standards required by legislation in each country. Examples of this are the ‘Meat Inspection Act’, the ‘Health of Animals Act’ of Canada (Department of Justice, 1990) and the USDA code of Federal Regulations-Safe Commercial Transportation of Horses for Slaughter Act of 1995, in the USA (Department of Agriculture, Animal and Plant Health Inspection Service, 2001). Both Canadian regulations are general for all species, whereas in the USA ‘Safe Commercial Transportation of horses for slaughter Act’ is specific to slaughter horses (Stull and Rodiek, 2000).

Third, there are private standards for transport, which include self-regulation by industry itself generally termed “codes of practice”. These guidelines in Canada are developed by inputs from diverse groups involved or interested in farm animal welfare such as non-governmental organisations and consumer quality assurance schemes (NFACC, 1998). The recommended code of practice for the care and handling of farm animals was promoted by the NFACC which also includes the national guidelines for transportation of horses in Canada.

In Canada, The Health of Animals Act and Regulations covers humane treatment of animals during transport. Penalties are enforced upon a violator by the Agriculture and Agri-Food Administrative Monetary Penalties Act and Regulations. Some of the regulations need updating as a consequence of increased scientific understanding and review takes place for continuous improvement (Doonan *et al*., 2009). For example, the requirement for rest and the feeding and watering patterns during long journeys are particularly important areas which need amendment. Health of Animals Regulation of Canada permits equines to be transported for 36 hours without food and water. Duration of transport has been much debated among the scientific community and there are studies, which have shown that horses cannot travel for longer than 24 hours in hot and humid conditions (Friend *et al.*, 1998, Friend, 2000, Smith *et al.*, 1996a) without showing welfare problems. Furthermore, Health of Animal Regulation of Canada does not stipulate specific stocking density requirements for road transport. It merely states that animals should not be so crowded that injury is caused. Some of the differences in regulations regarding transport between the European Union Regulations, the OIE Regulations and Canadian Regulations are shown in Table 1.5.

In the USA, United States Department of Agriculture (USDA) is responsible for the enforcement of "Safe commercial transportation of horses for slaughter Act of 1995" for the slaughter horse industry (including horses shipped to Canada). This Act was based on the evidence from the scientific community (Stull, 2001). Three separate research teams evaluated the risk factors which could affect the welfare of horses transported for slaughter such as duration of journey, water deprivation, trailer design and stocking density. Two salient aspects of this Act and subsequent regulations which followed are to phase out two-tier trailers and utilization of the shipper certificate (Department of Agriculture, Animal and Plant Health Inspection Service, 2001).

Table 1.5: Differences in regulatory guidelines regarding horse transport between the European Union (The European Council, 2005), the OIE (World Organization for Animal Health (OIE), 2010) Canada (Department of Justice, 1990) and the USA (Commercial transportation of equines for slaughter).

Category	**European Union Regulation ((EC) No 1/2005))**	**OIE regulations (Terrestrial Animal Health Code)**	**Canadian Regulation (Health of Animals Act)**	**USA Regulation (Commercial transportation of equines for slaughter)**
Duration of transport without feed, water and rest	Eight hours only. Specific needs such as food, water, space allowance and rest have to be met, if extended until 24 hours.	The amount of time animals spend on a journey should be kept to the minimum.	36 hours as per Health of Animals Regulation. 24 hours as per NFACC guidelines.	28 consecutive hours of transit without feed and water
Training needs for handlers and driver	Livestock handling training is a prerequisite from approved organisations	All individuals, including veterinarians, involved in transporting animals and the associated handling procedures should receive appropriate training and be competent to meet their responsibilities.	No specific regulations. Recommends self-regulation by industry. For example, livestock handling training provided by the employers.	No specific regulations.
Feed and water provision during transport	Horses should get access to feed and water, every 8 hours.	If journey duration is such that feeding or watering is required, access	Horses can be confined without feed or water for 36 hours.	Animals can be confined without feed or water for 28 hours. Equines

		to suitable feed and water for all the animals.		should have access to food, and water 6 hours prior to transit
Space allowance	1.75 m^2 for adult horse, 1.2 m^2 for young 6-24-month olds	As per relevant national or international document.	No specifications. Although 'code of practice' recommends 1.4 m^2 per adult horse	No specific regulation. Adequate space during transit to prevent injury or discomfort
Ramp angles	Not steeper than 20 degrees	No specifications.	The slope shall not be greater than 45 degrees.	No specifications
Multi deck vehicles	Only on the lower deck with no animals on the upper deck, Minimum head room: wither height +75 cm	No specifications	Each animal should be able to stand in its natural position without coming into contact with a roof.	Double-deck trailers should not be used for transport of horses to slaughter.
Grouping while transport	Individual stalling of horses recommended. Unbroken animals can be in groups of four.	Individual stalling of horses recommended. May be transported in compatible groups.	An equine shall, unless its hind feet are unshod be segregated from other equines during transport.	Segregation of stallions or other aggressive equines
Temperature monitoring inside the vehicle	Vehicle should be equipped with temperature monitoring system.	Animals should be protected against harm from hot or cold conditions during travel. The environment within vehicles in hot and warm weather can be regulated by the flow of air produced by the movement of the vehicle.	No specifications.	No Specification

1.4. WELFARE ISSUES ASSOCIATED WITH LAIRAGE AND SLAUGHTER

The lairage area of a slaughter plant has two important functions; first as a basic holding area for animals awaiting slaughter and second as a place where animals can be rested. Apart from this, it provides facilities for identification and ante-mortem inspection of animals. Under ideal conditions, lairage can help the animals recover from the stress suffered during transport (Warriss, 2003). Conversely, if not properly managed, it can create additional stress for animals. The amount of time that the animal spends in the lairage is a significant factor which needs to be managed. In the lairage, animals can go through psychological stress due to changes in environment, social disturbances and physical stress due to handling. The potential for horses to experience food deprivation, fatigue, and pain (if they are injured previously) while in the lairage (Terlouw *et al.*, 2008) has also been highlighted by previous studies.

After lairage, horses are moved to the stunning area for stunning, which involves some amount of handling. Micera *et al.* (2010) studied stress response of horses during lairage and slaughter and found that horses showed significantly higher plasma cortisol and catecholamine concentrations after stunning when compared to samples taken in the lairage. This finding suggests that there are some risk factors for welfare associated with the handling before stunning and/or the slaughter procedure itself, which was stressful to the horses. The horse slaughter industry and governmental agencies had recognised the importance of this welfare issue (handling stress) and responded by introducing regulations such as "complete stoppage of electric goad/prod usage for handling of slaughter horses”. There is a preference at some slaughter plants for using “gunshot” as a method of stunning instead of captive bolt to reduce handling of horses during stunning. As the damage to the brain is more extensive, the precision required for gunshot is not as exact as that required for a captive bolt and therefore head restraint may not be required. However, there is a need for more scientific work to be carried out in this area looking at the difference in response among horses stunned by gunshot and captive bolt.

1.4.1. Management during lairage

The Terrestrial Animal Health code of the OIE recommends adequate provision of resources during lairage for the animals to get rest (which includes adequate space), feed, water and protection from aggressive behaviour from other horses. These provisions can allow the horses to recover from dehydration and fatigue suffered during transportation. However, if horses are injured during transport and also if provisions are not adequate in the lairage, increase in the duration of stay in the lairage can be harmful to the welfare of the animal. To provide the animals with adequate facilities in the lairage effectively, there is a need for proper planning and designing of the lairage. This can be a problem, particularly when the slaughter plant is old and was built at a time when less importance was given to animal welfare. If the following factors are kept in mind while building a lairage facility the welfare of horses intended for slaughter might be improved.

1.4.1.1. Lairage capacity

Insufficient lairage capacity may result in animals having to wait in vehicles or may lead to overstocking within the lairage. The lairage area should be matched with the throughput of the slaughter plant. Over stocking may occur when lairage capacity has not been increased in line with increased throughput. High stocking density can lead to heat stress in hot weather and prevent animals from resting owing to interference from their conspecifics (Weeks, 2008). Each animal should have room to stand up and lie down and when confined in a pen, to turn around, except where the animal is reasonably restrained for safety reasons (e.g. fractious animals). Animals penned at high stocking density may not only suffer physical restriction, but also get reduced access to feed and water. The lairage should have sufficient accommodation for the number of animals intended to be held.

1.4.1.2. Lay out, flooring and gates

Terrestrial Animal Health Code of the OIE has detailed recommendations regarding design of the lairage. The layout of the lairage should be such that horses can be moved around the lairage and moved for slaughter with minimum handling of the animal. The optimum outcome in terms of lairage layout is to be able to move animals from the unloading point to the holding pen and on to the point of slaughter as directly as possible. The number of turns and corners should be minimal, and the route that the animals take should encourage forward movement. One-way gates, run-through lairage pens and elimination of right-angled corners in the system are just some of the points to be remembered while designing lairage. Such small details could have a significant effect in terms of efficiency of handling of animals, worker safety, and slaughter plant throughput while at the same time markedly improving animal welfare. The gaps between pen bars should be narrow enough to avoid legs getting trapped and possibly broken. It is also important to build some separate individual pens for adequate separation of fractious animals (e.g. some stallions) that may be prone to fighting (Farm Animal Welfare Council, 2003).

The floor surface should minimize risk of slipping for horses during movement in the lairage. Provision of anti-slip flooring can cause conflict of interest at the slaughter plant management level, as the floor must also be easy to clean for meat hygiene reasons. So a balance might be stuck between nonslip flooring and also a floor which can be easily cleaned regularly. It has been noticed that when welfare assessments were performed to evaluate risk in the lairage area, it could be difficult to separate the effects of poor flooring, lairage design and animal handling practices. For example, an animal rushed around a tight corner, even on a floor surface with a good foothold is at risk of slipping (Farm Animal Welfare Council, 2003) which shows the importance of having good handling procedures along with good non-slip flooring.

1.4.1.3. Noise

Noise can result in physiological and psychological stress responses in some species of animals (Ellen Kanitz *et al.*, 2005; Sundaramahalingam Manikandan *et al.*, 2006; Weeks, 2008). More scientific work needs to be carried out regarding the noise level in lairage, specifically for horses. The source of noise in any lairage or handling area could be created by animals, people, machinery and equipment (Weeks *et al*. 2012). Farm Animal Welfare Council, UK has identified noise as a potential stressor, particularly in the lairage area and has recommended the following:

- Separation of the lairage from the pre-slaughter handling area either by space or a soundproof wall or partition reduces the effect of animal noise on animals in the lairage.
- Attempt must be made to dampen sources of noise or make use of more suitable materials to reduce noise levels.
- Particular attention should be given to gates and a ‘non slam’ culture should be developed.
- Other sources of noise such as air lines and compressors can be reduced or eliminated by correct maintenance.
- Maximum noise levels and exposure limits should be set for animals as well as for operators (Farm Animal Welfare Council, 2003).

1.4.1.4. Ventilation

The range of ventilation in the lairage must be sufficient to control levels of toxic or irritant gases such as carbon dioxide and ammonia and to remove excess heat and humidity (Weeks, 2008). Ventilation should be able to cope with the range of expected climatic conditions and number of animals that the lairage will be expected to hold (World Organization for Animal Health (OIE), 2010). All slaughter facilities would benefit from a regular assessment of the lairage ventilation system. Guidelines for minimum and maximum temperature, humidity and level of ammonia in the lairage need to be specified (Farm Animal Welfare Council, 2003) after appropriate scientific studies.

1.4.1.5. Space allowance

Appropriate space allowances for animals to be slaughtered are yet to be specifically determined for lairage. The longer animals stay in lairage, the more need there is for rest. Therefore increased space allowance needs to be considered when the lairage time increases, so that animals are able to get up and lie down and lie undisturbed (Weeks, 2008). Horses will benefit if they have sufficient space to stand up, lie down and turn around without difficulty when penned. A search by the author in common scientific databases such as EBSCO, science direct and Google scholar did not yield any studies on horses that specifically relate to the optimum space allowances within a slaughter plant and thus slaughter plant operators and enforcement officers rely on figures taken from a wide variety of sources, including transport guidance and the welfare codes of recommendations. The problem can be compounded by space use in the lairage due to the need to avoid mixing some groups of animals (e.g. stallions), the wide variation in journey time to the slaughter plant (hence animals arriving at different time points) and animals being penned for durations ranging from an hour to overnight (Farm Animal Welfare Council, 2003).

1.4.1.6. Social grouping

Mixing unfamiliar groups of animals in the lairage may result in aggression among animals (Hartmann *et al.*, 2009), and meat quality problems due to increased bruising. Research in cattle has demonstrated that mixing animals can lead to a greater incidence of aggression, although in practice, cattle are more likely to be mixed both on the trailer and at the lairage (Smith *et al.*, 2004) than horses. Increased prevalence of injuries was reported in horses, which are housed in groups compared to horses stabled separately for day to day management practices (Fürst *et al.*, 2006). Group size should be limited and horses should be kept in farm groups all the way through the transport, marketing and slaughter process. Mares with a foal at foot should be kept together. Stallions need separation from other horses.

1.4.1.7. Feeding and watering

There are concerns that increased duration of transport and marketing times for horses could result in long periods without feed or water. Legislation and guidance must take account of the fact that animals may undergo a period of transport and possibly time in a market or collection centre before they are slaughtered. Most countries have guidelines, which state that water must be 'available' to all animals at all times. In practice, water availability may be restricted to animals through overstocking, insufficient watering points, contamination or if the drinking facility is not suitable for the species. Drinking water should always be available to the animals, and the method of delivery should be appropriate to the type of animal held. Troughs should be designed and installed in such a way as to minimize the risk of fouling by feces, without introducing risk of bruising and injury in animals, and should not hinder the movement of animals (OIE, 2012). Where feed troughs are provided, they should be sufficient in number and feeding space should allow adequate access to all animals to feed. The feed troughs should be placed in such a way that they do not hinder the movement of animals.

1.4.1.8. Time spent in lairage

The time any animal, or group of animals, spends within the lairage varies greatly, both between slaughter plants and between batches of animals within the same slaughter plant. The OIE's Terrestrial Animal Health Code recommends a maximum lairage of 12 hours. In a study in Chile by Werner and Gallo (2008), the mean lairage time for horses spent in lairage was 20 hours 6 minutes, which is considerably longer than the OIE recommendation. It was argued that the long lairage time allowed the animals to reduce blood lactate concentration, packed cell volume, glucose and cortisol concentrations and bring them close to the normal clinical range, indicating normalization in the stress response (Werner and Gallo, 2008). The Farm Animal Welfare Council (2003) recommends slaughter of animals soon after arrival to the slaughter plant, because increasing lairage time results in additional stressful situations created by noise and fear. An animal's ability to rest in the lairage is limited since it can take several days for the animal to adjust to the new environment and resume normal patterns of behaviour (Jarvis *et al*., 1996).

1.4.2. Management during slaughter

Method of stunning, handling of the animal during stunning and time taken for these operations are risk factors associated with the welfare of the animals before slaughter. The stunning method should be efficient to make the animal unconscious and should also involve minimum handling of the animal. Slaughter plant management must be careful about the length of time horses spend in the stunning box and the competence of the personnel doing stunning. Maintaining a high standard of welfare requires constant management attention and vigilance. Employees should remain calm and avoid rough handling. The risk factors which affect the welfare of animals during slaughter are described below.

1.4.2.1. Handling

From a welfare standpoint, animals should be handled as little as possible in lairage and also during stunning. Handlers should be competent and the systems used should be designed to encourage smooth movement of the stock. Benign handling devices such as flags, flappers and bags should be used instead of electric goads/prods or sharp objects. If goads/prods are used, it is important that handlers do not become overzealous in their application of these devices to the point where they may cause harm, or may use them inappropriately. Rough handling can increase the risk of animals slipping and falling during handling and stunning. One study reported that plasma activity of CK in cattle was positively correlated with the time spent in the race and associated handling (Cockram and Corley, 1991). Regular training of the staff involved in handling has proved to be a good tool to improve handling and stunning (Grandin, 2010a).

1.4.2.2. Method of stunning

Horses intended for slaughter are stunned by a captive bolt (Micera *et al.*, 2010) or shot using a rifle (Grandin, 1994). When properly applied, both cause trauma to the cerebral hemispheres and the brainstem, resulting in immediate unconsciousness (Werner and Gallo, 2008; Grandin, 1994). If exsanguination is performed immediately after stunning, then unconsciousness leads to a painless, humane death. Penetrating captive bolt instruments are powered by gunpowder or compressed air that provides sufficient energy to penetrate the horse's skull (AVMA, 2007). In Canada, some slaughter plants use a rifle shot for slaughter of horses while others use captive bolt. There are some studies carried out on rifle shot as a method of stunning which suggest that if performed properly, death is immediate (Blackmore *et al.*, 1995). The optimal site of penetration of the horse's skull is one-half inch (4 cm) above the intersection of two diagonal lines: one diagonal line extends from the base of the right ear to the lateral corner of the opposite eye and the other line extends from the base of the left ear to the lateral corner of the opposite eye (OIE, 2012). The firearm should be aimed directly down the neck, perpendicular to the front of the skull and held at least 2 to 6 inches away from the point of impact. A 0.22-caliber long rifle is adequate, but 9-mm or 0.38-caliber pistols have greater penetrating potential. If a shotgun is the only available firearm, a rifled slug is preferred (Werner and Gallo, 2008).

A significant welfare issue in the slaughter plant could be horses not being stunned properly. The numbers of stunning attempts needed to stun the animal and number of animals sensible or conscious after exsanguination are two variables studied to assess stunning efficiency. The Werner and Gallo (2008) study in Chile showed that only 85% of horses became unconscious on the first attempt of stunning and the remaining 15% needed a second attempt which could be attributed to improper stunning practises. The same study reported that 57.2% of horses stunned returned to consciousness. However, there are no published studies on the efficiency of horse stunning practices in North America.

Micera *et al.* (2010) showed significant elevation of plasma cortisol, epinephrine and nor-epinephrine concentrations in blood collected by venepuncture after stunning procedure than that in blood collected 45 minutes before stunning, thereby indicating the stressful nature of stunning. Another similar study found that physiological variables such as blood lactate concentration, blood glucose concentration, plasma creatine kinase activity, cortisol and PCV were significantly higher after stunning compared to pre-stunning (Werner and Gallo, 2008). At this point, it is hard to differentiate whether these physiological variables are responding to the stunning procedure itself or whether their increase is due to a stressful situation such as handling during the stunning process. However, these studies indicate the importance of a scientifically evaluated method of stunning in order to reduce the risk of poor welfare in horses. Unfortunately, there is no scientific literature comparing the efficiency of captive bolt and rifle for stunning horses.

When horses are shot, the noise created can induce fear in the other horses waiting to be stunned or shot. Noise can be an issue both with captive bolt and rifle shot. Gregory *et al*., (2007) suggested that the captive bolt (possibly air driven captive bolts) produces less noise (less than 111dB), but was also associated with a shallow depth of concussion in cattle. So it is important to strike a balance.

Handling before stunning is also an important procedure which can have an impact on the welfare of horses during slaughter procedure. Training the personnel involved in handling of horses during stunning about flight distance of horses and practical ways to make them move forward without evoking a negative response are good strategies to improve welfare.

1.4.2.3. Time taken for stunning and exsanguination

Time taken for stunning and exsanguination procedures has a significant effect on the welfare of the animal. Generally, the length of time an animal spends in the stunning box is related to the speed of the slaughter line which in turn depends on the speed and efficiency of operators (Werner and Gallo, 2008). When these variables were recorded in a horse slaughter plant in Chile, mean time in the stunning box was 9 minutes and 48 seconds (Werner and Gallo, 2008). Even though, the Meat Inspection Act and associated Regulations of Canada do not specify any limits for the time taken between stunning and exsanguination, research has shown that cattle can in some circumstances, regain consciousness after stunning with captive bolt (Appelt and Sperry, 2007) and hence exsanguination has to be carried out within a minute to reduce this chance. It is relevant to note here that United Kingdom regulations allow only 30 seconds between stunning and exsanguination for bovines and 20 seconds for smaller animals, such as sheep goats and pigs (Government of United Kingdom, 1995) however, there was no time limit mentioned about horses in this legislation.

1.5. ASSESSMENT OF WELFARE IN LAIRAGE AND SLAUGHTER

1.5.1. Assessment in lairage

Lairage is a critical control point in food animal slaughter activity in terms of animal welfare. The risk factors which affect welfare in the lairage are design of the lairage which includes capacity, layout flooring, type of gates, ventilation, noise levels and resource factors such as stocking density, feeding and watering and time spent in lairage (Farm Animal Welfare Council, 2003). Scientific work on most of these risk factors is limited and ought to be carried out, if minimum specifications are to be understood (Algers *et al.*, 2009). Research studies are needed to lay down minimum specification for different species including horses. Once minimum specifications are put in place, they can be enforced by regular inspections. Assessment of either new or existing premises should take into account the need for the capacity of the system to cope with incidents such as slaughter line breakdown. In Canada, welfare assessments are the responsibility of the slaughter plant and effectiveness is monitored by Canadian Food Inspection Agency (CFIA) inspectors (CFIA, 2012). Apart from the resource factors, animal-based assessments such as the percentage of animals slipping; vocalization, biting and kicking frequency can also be quantified and used as a baseline for further improvement of welfare. Vocalization has been used as an assessment variable in cattle and pigs to detect welfare issues in the lairage area (Grandin, 2010a; Weeks, 2008). It can also be used in horses although scientific evidence is lacking on the validity of using vocalization as a welfare assessment variable in horses. The idea of scoring the number of slips and falls by animals at key points is a good practical method for both slaughter plant operators and enforcement officers to make an objective assessment. Grandin (2010a) indicated that more than a few percentage points of animals slipping or falling are unacceptable (Grandin, 2010a). The slaughter plant management can make sure that high standards of welfare is maintained by using these behaviours such as the number of slips and falls by animals as assessment variables and training people to look for these behaviours.

1.5.2. Assessment during stunning

Management procedures during stunning have a significant impact on the welfare of the animal intended for slaughter. Werner and Gallo (2008)'s study on horses slaughtered in Chile showed that blood lactate concentration rises significantly during the stunning procedure, which indicates high muscle activity. This increased muscle activity could be associated with a fear response shown by horses. Assessment of slaughter activity in terms of animal welfare concerns could be performed by quantifying some variables which Grandin *et al.* (1998) used extensively in livestock other than horses. Some of the important variables Grandin (1998) used are

- Percentage of animals stunned with one captive bolt or firing shot.
- Percentage of animals slipping, falling during handling and stunning.
- Percentage of animals vocalizing during handling and stunning.
- Percentage of animals conscious on the bleeding rail.
- Amount of time spent in the stunning box.
- Amount of time taken after stunning before exsanguination.

Grandin (2010c) after studying captive bolt stunning suggested that 99% of animals should be stunned in the first attempt. Behaviour variables such as the presence of a righting reflex while on the bleeding trail and eye movements when touched after stunning (Grandin, 2010b) provide information on inefficient stunning procedures, which have major welfare significance because the animal is still conscious in order to exhibit these signs.

To assess slaughter procedures Werner and Gallo (2008) used most of above variables elaborated by Grandin (1998) in Chilean slaughter plants where horses were slaughtered. To assess the stunning procedure; rhythmic respiration, eye movements, vocalization, head elevation and attempts to stand were used. Welfare assessment protocols should be developed in Canadian slaughter plants to establish baseline values as currently there are no studies to reflect North American perspectives. Thereafter, continuous improvements can be made to improve welfare.

1.5.3. Carcass assessment

If slaughter animals are handled intensively before, during, or after transport and if they are grouped together in unfamiliar groups, this could lead to bruising of some of the carcasses (Costa *et al.*, 2005; Costa *et al.*, 2006; Jago *et al.*, 1996). Bruising indicates injury to the animal during the transport and slaughter procedure and hence is a good indicator of injury along the chain of events. When the carcass is dressed, size, colour and site of every bruise could be recorded. An identification of the region where bruising is commonly occurring, could provide a clue to the risk factors associated with the bruising.

1.5.4. Slaughter-related regulations

In Canada, the Meat Inspection Act and Regulations provides protection for all species of animals slaughtered for human use in federally approved slaughter plants, including slaughter horses. At the international level, there are also other regulations to protect animals from suffering during the slaughter procedure. The European Union has directives that the European Union countries have to follow and OIE also provides additional international guidelines. There are significant differences in the regulations between European Union, OIE and Canadian regulations to protect slaughter animals from suffering (see Table 1.6) during the slaughter procedure.

Table 1.6: Differences in regulatory guidelines regarding horse slaughter procedures between European Union, OIE and Canada.

Category	**European Union Directives**	**OIE regulations**	**Canadian regulations-** Meat Inspection Act
Use of electric goads/prods	The use of instruments that administer electric shocks shall be avoided as far as possible.	Goads/prods should not be used in horses.	No goad/prod shall be applied to the anal, genital or facial region of the food animals.
Ventilation and space allowance	Ventilation systems shall be designed, constructed and maintained, taking into account the expected range of weather conditions.	Forced ventilation or other cooling systems may be necessary under certain conditions to avoid build-up of temperature and humidity.	Every food animal in a holding pen awaiting slaughter shall be provided with adequate ventilation.
Feeding and watering	Food animals for slaughter should not suffer from prolonged withdrawal of feed or water.	Animals which have not been slaughtered within 12 hours of their arrival should be fed at appropriate intervals. Water should be available always.	Every food animal in a holding pen awaiting slaughter shall be provided with access to potable water and shall, if held for more than 24 hours be provided feed.
Stunning effectiveness	Operators will be required to evaluate the efficiency of their stunning method through animal based indicators.	No specifications	Every food animal that is slaughtered has to be rendered unconscious before bleeding.
Waiting time in lairage	No specification for waiting periods in lairage	Waiting time should be minimised and should not exceed 12 hours.	No specification for the waiting period in lairage
Assessment of personnel for Handling/ Stunning	Certificate of competence is needed	Competence may be gained through formal training and/or practical experience.	No specifications

Assessment of welfare	Each slaughter plant will have to appoint an Animal Welfare Officer who will be accountable for implementing the animal welfare measures. This will complement regular official inspection.	Resource-based measures and outcome-based measures (e.g. bruises, lesions, behaviour, and mortality) should be used to monitor the level of welfare of the animals.	No specifications

1.6. RESEARCH OBJECTIVES

The overall objective of this project is to identify the welfare issues and associated risk factors caused by transportation of horses to slaughter plants.

The specific objectives of this project are:

1. Quantifying the types of vehicles used by the horse slaughter industry in Canada, USA and Iceland.

2. Identifying source of origin of horses and journey duration of horses transported to slaughter plants in Canada and Iceland.

3. Assessing the welfare of horses on arrival at the slaughter plant in Canada and Iceland.

4. Examining relationships between journey characteristics and the welfare assessments.

5. Assessing the prevalence of pre-existing conditions identified post-mortem that might have been associated with pain or discomfort before transport.

6. Evaluating the methods of welfare assessment associated with transport and slaughter of horses at a slaughter plant.

7. To develop recommendations for amendments to guidelines and legislation to protect the welfare of horses and procedures for monitoring of horse welfare and enforcement of legislation.

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CHAPTER 2

AN ANALYSIS OF USDA OWNER/SHIPPER CERTIFICATES TO DESCRIBE THE TRANSPORT OF HORSES FOR SLAUGHTER FROM THE UNITED STATES OF AMERICA TO CANADA IN 2009

2.1. **INTRODUCTION**

In Canada, the transport of horses for slaughter involving long distances and durations has recently been a subject of public debate, thereby forcing the horse industry and enforcement agencies to develop strategies to tackle this issue (Alberta Farm Animal Care, 2008). Societal disapproval regarding horse slaughter for human consumption in the USA resulted in federal and state regulations that effectively stopped the slaughter of horses for human consumption within that country (Stull, 2012). This led to horses being shipped to neighbouring countries and precipitated an increase in the number of horses from the USA that were slaughtered in Canada and Mexico (United States Government Accountability Office, 2010). Quantitative data regarding journey characteristics after this restrictive regulation on slaughter of horses in the USA came into effect are limited. In this chapter, data from owner/shipper certificates obtained from the United States Department of Agriculture (USDA) for the year 2009 were reviewed, analysed and discussed.

Database of The Food and Agricultural Organisation (FAO) indicate that a considerable percentage of horses slaughtered in Canada originate from the USA. FAO statistical database reported that 120,000 horses were slaughtered in Canada in 2009 (FAO statistical database, 2009). However, Agriculture and Agri-Food Canada reported that the number of horses slaughtered in 2009 was 93,946 horses (Agriculture and Agri-Food Canada, 2009). In the same year, 110,000 horses were dispatched for slaughter from the USA to Canada and Mexico (FAO statistical database, 2009). According to the Equine Welfare Alliance data in 2009, 57% of horses intended for slaughter from the USA were slaughtered in Canadian slaughter plants, 42.8% in Mexican, and 0.2% in Japanese slaughter plants (Equine Welfare Alliance, 2013). Since 57% of horses intended for slaughter from the USA were slaughtered in Canada (as reported by Equine Welfare Alliance), a study of the transport patterns of slaughter horses from the USA to Canada will provide useful information to understand the potential welfare implications of journeys of slaughter horses from the USA to Canada.

Owner/shipper certificates (hereafter called shipper certificate) were introduced by the USDA in 2001 with an aim to enforce humane transport of horses for slaughter (United States Government Accountability Office, 2010). All horses originating from the USA that are destined to be slaughtered are required to be accompanied by a completed USDA shipper certificate. Hence, it was decided to use this source (the shipper certificate) as the data for this study. Information regarding the number of horses transported, source of journey, destined slaughter plant, journey duration, animal characteristics such as breed and sex, and basic health status details of horses transported were obtained from these certificates.

The shipper certificate was originally designed as a trace-back tool to investigate and document violations of the “Commercial Transportation of Equines to Slaughter” program (USDA, 2011). These certificates are signed by the owners/shippers after inspection of the horses before transporting them for slaughter. After transport, these certificates are collected by the officials in the host country, i.e. at the slaughter plant in Canada, and returned to the USDA veterinary services. The main goal of these shipper certificates is self-regulation by the owners or shippers regarding the fitness of horses sent for slaughter, so that they are in compliance with the USA's federal regulations regarding commercial transportation of equines to slaughter. The regulations regarding commercial transport of equines to slaughter prohibit the transportation of a horse that is:

a. unable to bear weight on four limbs,
b. unable to walk unassisted,
c. blind in both eyes,
d. a foal under 6 months of age, and
e. a pregnant mare that is likely to foal (give birth) during the trip (USDA, 2011).

The objective of this study was to analyse the entire shipper certificate data for 2009, in order to provide quantitative information about the journey and animal characteristics of horses transported for slaughter from the USA to Canada, specifically:

- The origin and endpoint of the journeys
- The number of each sex and breed or type of horses within each load
- The declared journey duration on the shipper certificates.

2.2. MATERIALS AND METHODS

Through the Freedom of Information Act (FOAIQ) request no. 2011-00300-F, all records of shipper certificates (Veterinary Services FORM 10-13) for the year 2009, particularly horses transported from the USA to Canada, were requested from the USDA. In response to this FOAIQ request, the USDA released 4400 records pertaining to transport of horses for slaughter. At the time of request, 2009 was the last year for which complete data was available. Even though the USDA indicated that they had released 4400 records in response to this FOAIQ request, the data corresponded to only 2200 consignments/truckloads (hereafter called consignments) of horses. This discrepancy was because each completed shipper certificate form consisted of two pages. Among the 2200 records of consignments released, there were some health reports prepared by the border inspection agency that contained repetitive information and were not considered further. Moreover, for this study, records pertaining to transport of horses to the Mexican border were excluded, as the focus was on transport of horses from the USA to Canada. After both of these exclusions, records pertaining to 1800 consignments were used for this analysis. Information on origin of journey, the destination slaughter plant, journey time, number of horses transported in each consignment, sex ratios and breed composition were obtained from these forms. However, not all of the information on each form was available, partly due to incomplete information provided by the owner/shipper or the Canadian Food Inspection Agency (CFIA) as well as the USDA blocking information due to privacy rules.

The USDA had categorised the origin of horses for slaughter in the USA by defining them into assembling point, feedlot and stockyard. Detailed descriptions regarding the definitions of origin of horses are as follows:

Assembly Point: Any facility, including auction markets, ranches, feedlots, and stockyards, in which equines are gathered in commerce.

Feedlot: Any facility which consolidates livestock for preconditioning, feeding, fattening, or holding before being sent to slaughter.

Stockyard: Any place, establishment, or facility commonly known as stockyards, conducted, operated, or managed for profit or non-profit as a public market for livestock producers, feeders, market agencies, and buyers, consisting of pens, or other enclosures, and their appurtenances, in which live cattle, sheep, swine, horses, mules, or goats are received, held, or kept for sale or shipment in commerce.

Data were entered into a spreadsheet (Microsoft Excel, Microsoft Corporation, One Microsoft Way Redmond, WA 98052-7329) and transferred to statistical software's (Minitab 15, Minitab Inc Quality Plaza 1829 Pine Hall Rd State College PA 16801-3008 USA and Stata 12.1,StataCorp, 4905 Lakeway, College Station, Texas 77845 USA) for descriptive analyses. Origin of journey and endpoint (slaughter plant) were tabulated to understand journey patterns. Journey duration was calculated from journey time provided in the certificates and distribution of consignments by month was calculated from the date of journey provided in the certificates. Journey duration was also calculated using Google Maps for each consignments for which origin of journey and endpoint are known so that comparisons could be made with actual journey duration derived from journey time.

2.3. RESULTS

2.3.1. Transport pattern of horses from the USA to Canada for slaughter

In 2009, there were six federally approved slaughter plants operating in Canada for the slaughter of horses. These slaughter plants were located in four provinces; one in Ontario (Norval meats, Proton Station), two in Québec (Richelieu Meat Inc., Massueville and Les Viandes de la Petite-nation Inc., St-André Avellin), two in Alberta (Bouvry Export Calgary Ltd, Lacombe and Fort Macleod, Alberta), and one in Saskatchewan (Natural Valley, Wolseley). As illustrated in Figure 2.1, horses were transported from most of the northern states of the USA (Washington State, Oregon, Idaho, Montana, South Dakota, Minnesota, Illinois, Indiana, Kentucky, Pennsylvania, Ohio, Iowa, New York and Maine) to these six slaughter plants.

Figure 2.1: The origins† of horses transported from various states of the USA (indicated in blue) and the six slaughter plants in Canada (indicated as pink).

† State specified in the shipper certificate for the origin of the journey to the slaughterplant in Canada.

Table 2.1 illustrates the distribution of consignments that arrived at the six different slaughter plants in Canada in 2009. The greatest percentage of consignments and the greatest number of horses transported for slaughter were to the Massueville (Québec) slaughter plant followed by Fort Macleod (Alberta), St. André Avellin (Québec), Lacombe (Alberta), Proton Station (Ontario), and (Wolseley) Saskatchewan.

Table 2.1: Number of consignments and number of horses received at the six federally approved slaughter plants in Canada in 2009.

	No. of consignments	% of consignments	No. of horses	% of horses
Slaughter plant				
Massueville, Quebec	746	41.4	21,931	42.1
Fort Macleod, Alberta	378	21.0	10,267	19.7
St. Andre Avellin, Quebec	350	19.4	10,404	20.0
Lacombe, Alberta	141	7.8	4,233	8.1
Proton Station, Ontario	122	6.8	3,423	6.6
Wolseley, Saskatchewan	63	3.5	1,878	3.6
Total	1,800[a]	100.0	52,837	100.0

[a] No. of shipper certificates where the destination was identifiable.

Table 2.2 illustrates the distribution of consignments to Canadian slaughter plants according to month of transport. The table shows that horses were sent for slaughter throughout the year, although a trend of reduction can be noticed in the last few months of the year.

Table 2.2: Distribution of horse consignments transported for slaughter by each month of the year as obtained from the owner/shipper certificates.

Month of year	No. of consignments	% of consignments
January	106	15
February	63	9
March	72	10
April	51	7
May	51	7
June	53	7
July	59	8
August	50	7
September	90	12
October	46	6
November	48	7
December	37	5
	726	100

[a]Number of consignments were date of loading is identifiable (not blocked for privacy reasons)

2.3.2. Origin of journey

Horses were transported from 16 northern states of the USA to the six federally approved slaughter plants in Canada. The origin of the journey specified on the shipper certificates was categorized as an auction market, horse collection centre or feedlot (as specified by stakeholders). However, this information was blanked out by the USDA on 827 certificates. Out of the consignments for which origin of journey was made available 32% were from auction market, 33% from feedlots and 35% from horse collection centres. Table 2.3 describes the origin of the journey specified on the owner/shipper certificates, number and percentage of consignments from different states of the USA into Canada and the associated number of horses. More than 50% of the consignments arrived from Montana, Iowa and Ohio.

Table 2.3: Distribution of consignments[a] in 2009 according to origin of journey from data obtained from certificates for which information was available

State	Source [b]	No. of consignments	% of consignments	No. of horses	% of horses
Montana	Feedlot	287	33.3	7,709	30.8
Iowa	Collection Centre	129	15.0	4,029	16.1
Ohio	Auction	25	2.9	728	2.9
	Auction	81	9.4	2,374	9.5
Oregon		98			
Kentucky	Auction	65	7.5	1,984	7.9
	Auction	10	1.2	311	1.2
Washington	Collection Centre	74	8.6	2,100	8.4
Indiana	Auction	5	0.6	138	0.6
	Auction	59	6.8	1,770	7.1
Minnesota	Collection Centre	34	3.9	1,024	4.1
New York	Collection Centre	33	3.8	1,033	4.1
Pennsylvania	Auction	25	2.9	787	3.1
	Auction	2	0.2	61	0.2
South Dakota	Auction	6	0.7	178	0.7
	Collection Centre	16	1.9	522	2.1
North Dakota		12			
Maine	Collection Centre	5	0.6	99	0.4
Idaho	Collection Centre	5	0.6	124	0.5
Michigan	Auction	1	0.1	34	0.1
Illinois		1			
Total		973	100	25,005	100

[a] Using data obtained from 973 certificates for which information on state of origin was available. Most sources were blanked out on the shipper certificates. The source category and state were identified using the information available on the certificate and by searching the internet.

[b] In some states there was more than one source.

2.3.3. Journey duration

Journey duration was calculated from the shipper certificates that had four relevant pieces of information: time at which horses are loaded on conveyance, date of loading, date of inspection by CFIA official at destination and time of inspection at destination. Journey duration was calculated under the assumption that the CFIA officials inspected the consignments immediately after unloading, which may not always have been the case. In 1421 shipper certificates, time of loading and time of unloading were blocked. Analysis of the limited available journey duration data (379 out of 1800 consignments {21%} in Table 2.4) indicated that it ranged from 3.25 to 105 hours. Due to the unreliable nature of the journey duration data, an attempt was made to assess whether the calculated journey duration appeared to be reasonable by using Google Maps to calculate the estimated direct journey duration from the origin of the journey to the slaughter plant. Journey duration determined by Google Maps ranged from 5 hours to 24 hours under normal driving conditions from the place of origin to the destination slaughter plant. Comparative estimates between these two methods of journey duration calculation are provided in Table 2.4.

Table 2.4: Comparison between declared journey duration calculated from the owners/shippers certificate and journey duration estimated through Google Maps default settings.

Journey duration	**n**	**Min**	**Q1**	**Median**	**Q3**	**Max**
Declared journey duration (h) obtained from owners/ shippers certificate	379	3.25	4.5	19	27	105
Journey duration (h) estimated by Google maps from the origin to the slaughter plant		5	8	11	19	24

Table 2.5 illustrates the different states of the USA from which each of the Canadian slaughter plants received horses for slaughter and their corresponding median journey duration and range in hours. Table 2.6 illustrates the percentage of horses transported to a Canadian slaughter plant in 2009 by journey duration categories as declared on the shipper certificates.

Table 2.5: Journey duration to each slaughter plant calculated from the information on the shipper certificates.

Slaughter plant location	State from which consignments originated	Median journey duration (h)	Journey duration (Range in h)
Lacombe, Alberta	Minnesota, Montana and Idaho	23	12-76
Fort Macleod, Alberta	Montana, Washington State, Minnesota and South Dakota	4.5	3-50
Massueville, Québec	Maine, Pennsylvania, Kentucky and Indiana	30	7-105
Proton Station, Ontario	Minnesota, Iowa, South Dakota, Indiana and Ohio	20	7-63
Wolseley, Saskatchewan	South Dakota and Indiana	7	6-19
St. André Avellin, Québec	Michigan, Ohio, Pennsylvania and Iowa	27	5-69

Table 2.6: Percentages of horses transported to a Canadian slaughter plant in 2009 by journey duration categories (as declared on the shipper certificates).

Duration of journey (h)	Number of consignments	% of consignments	Number of horses	% of horses
<6	139	37	3672	35
6<12	26	7	709	7
12<18	15	4	351	3
18<24	48	13	1303	13
24<30	71	19	2066	20
30<36	25	7	703	7
36<42	20	5	614	6
42<48	13	3	376	4
>48	22	6	619	5
Total	379	100	10,413	100

2.3.4. Horse characteristics

Of the 1800 consignments recorded on the certificates provided, there were 52,837 horses transported in total from the USA to Canada. Quarter horses accounted for 74%, thoroughbreds 6%, draught horses 5%, ponies <1% and miscellaneous category accounted for 15% of the total horses. The miscellaneous category mostly comprised of standard bred horses. Fifty-nine percent of the horses transported were mares and 40% were geldings. Only 1% of the horses were stallions.

The median number of horses in each consignment was 29 (range of 13 to 41 horses). Some owners entered detailed comments regarding fitness of the horses in the shipper certificate, such as blind in one eye or lame in one leg. However, the terms used by the owner/shipper to denote health issues were vague (e.g. dead left eye), which might have been used to describe a blind left eye. These comments were available in only a few forms and hence they were not summarized. In total, there were only two forms with identifiable CFIA official comments regarding dead-on-arrival horses along with some other comments regarding health issues. One of the dead-on-arrival recordings was to St. André Avellin slaughter plant, for which the journey duration details were blanked out. In the only other shipper certificate form which indicated a dead-on-arrival case, all details regarding source of journey, destination and journey duration were blocked.

2.4. DISCUSSION

This study provided information on journey characteristics and animal characteristics of horses transported for slaughter in 2009 from the USA to Canada. The data obtained from the USDA by FOAIQ request, accounted for about 84% of the total horses transported for slaughter from the USA to Canada (52,837out of 62, 700) in 2009 (as reported by Equine Welfare Alliance). This demonstrates that the sample population included in this study accounted for most of the horses transported that year. However, the reliability of the data on some aspects, particularly the journey duration and origin of journey was less reliable as it was influenced by the USDA-blocked data for privacy issues and other factors such as incomplete information. While generalizing the results of this study, it is essential to realise that, as of today, the number of federally approved slaughter plants for horse slaughter has been reduced to four because of the closure of the Saskatchewan and Ontario slaughter plants. Further, shipper certificates do not capture horses that are transported from the USA to Canadian feedlots and may be slaughtered after a period of time.

The declared journey duration data obtained from the shipper certificates indicated that there was a significant percentage (14%) of horses that may have exceeded the 36 hour journey duration limit set by the Health of Animals Regulations (Canada Department of Justice, 2012). There have been recorded instances in which CFIA officials had imposed monetary penalties on shippers who violated this limit. For example, in 2008 there was a case between “A60351 1301479 Ontario Inc. v. Canadian Food Inspection Agency” (obtained by RTA #60351**)** where the drivers who transported 27 horses from Brownton, Minnesota, to Viande Richelieu Meat Inc., in Massueville, Québec, which took 47 hours, were penalised 2000 Canadian dollars. Journey duration estimates from this study indicate that journey duration could be a potential welfare issue in horses transported from the USA to Canada for slaughter. Previous studies by Stull (1999) and Friend (2000) have identified journey duration as a potential welfare issue in horses intended for slaughter. These studies indicated that there was an increased risk of injury, dehydration and fatigue with increased journey duration. Chapter 6 also deals with the issue of journey duration and their associations with specific welfare problems. A critical aspect which needs to be considered while dealing with journey duration in slaughter horses is that the shipper certificate data and Chapter 6 data both capture only the last leg of the journey. In most cases a horse would have undergone transportation in order to reach the auction market, feed lots or horse collection centres mentioned in the shipper certificate.

Interpretation of the journey duration data in this study needs to be cautious as data pertaining to journey duration could be obtained from only 21% of the shipper certificates. In most certificates; this information was blocked for privacy reasons. Even from certificates where information regarding journey duration was available, it was difficult to determine accurate journey duration due to two issues. First, for the end-of-journey time and date, it was difficult to know whether the recordings made by the CFIA official were performed immediately after unloading of horses from the truck or during ante-mortem examinations (there could be a time lapse between the end of journey and ante-mortem inspection). Second, some consignments could have been unloaded during transit e.g. at the Michigan border (a major transit point for livestock between the USA and Canada). It was difficult to determine from the shipper certificate, if there were any rest periods during the journey.

Horses transported for slaughter from the USA to Canada have to comply with the USA federal regulation as stated in “Commercial Transportation of Equines to Slaughter”

(GPO Federal register, 2011) and also the Canadian "Health of Animals Regulations" pertaining to journey duration. There are two aspects of the Canadian "Health of Animals Regulation" which deals with journey duration, and with which transporters need to comply. Article 148 deals with "Food and Water for Animals in Transit" and Article 138 deals with "Sick, Pregnant and Unfit Animals". More specifically

Article 148 (1) (a) states that:

"No person shall confine in a railway car, motor vehicle, aircraft or vessel; equines, swine or other monogastric animals for longer than 36 hours without feed and water during transit".

Article 138(1) (b) states that

"No person shall load or cause to be loaded on any railway car, motor vehicle, aircraft or vessel and no one shall transport animals that has not been fed and watered within five hours before being loaded, if the expected duration of the animal's confinement is longer than 24 hours from the time of loading"

Regarding article 138 (1) (b) the owner or transporter who is shipping has to declare that the horses have had access to food, water and rest for a minimum of 5 consecutive hours immediately before loading into the conveyance. Both these Canadian regulations are complimented by the "Commercial Transportation of Equines to Slaughter" of the USA regulation which require that "the equines have access to food, water and the opportunity to rest for at least 6 hours prior to transit and following 28 consecutive hours or more of transit" (GPO Federal Register, 2011).

Comparing the journey duration recorded on the owner/shipper certificates and journey duration as estimated by Google Maps provides insight on two salient aspects.

1. When the distances were shorter (e.g. from the Pennsylvania to the Québec slaughter plant), the declared journey duration was shorter than Google Maps estimated journey duration indicating that the journey was completed faster than the default travel speed settings in the Google estimation.
2. Contrary to the short distances, when distances were longer, then the declared journey durations were much longer than the Google Maps estimation. This could be either due to a rest taken during transit, delay at the border or CFIA officials not recording the actual arrival time of the consignment at the slaughter plant. It is possible that the CFIA officials at the slaughter plant recorded the unloading time much later than when the consignment reached the slaughter plant (perhaps during ante mortem examination).

The owner/shipper certificate records obtained from the USDA provided very limited information on the origin of transport when compared to the destination details, which was always one of the six slaughter plants. This was partly due to the owner or shipper providing incomplete information regarding origin of journey in the shipper's certificate and also due to the USDA privacy rules. In many shipper certificate forms, the origin of journey was blocked due to privacy issues. Incomplete information on shipper certificates had been reported in an earlier review by the USA Congress in 2007 (United States Government Accountability Office, 2010). Table 2.3 illustrated the different states of the USA from which horses originated, obtained from available data. As information regarding the precise origin of journey (auction market, feedlot or horse collection centre) is not known, the states from which the consignment originated was given importance. An attempt was made using an internet search to locate the exact origin of each journey which are tabulated in Table 2.3 and 2.5. From each of these places of origin (states), many companies could have been involved in procurement and collection of horses for slaughter to put together a consignment. These procurement or collecting companies could be a horse collection centre, an auction market or a feed lot.

A majority of the consignments originated from the following three states of the USA; Montana, Ohio, and Iowa, which accounted for >50% of the shipments (Table 2.3). Much of the horses (60%) were shipped to the two slaughter plants in the Québec region of Canada, one of which was selected for our detailed study (Chapter 6). Transport occurred throughout the year and hence horses may need protection during extreme winter months and summer months while in-transit in terms of increased ventilation in summer and protection from cold wind drafts in the winter.

The animal characteristics determined from this study point out some interesting statistics. Only one percent of horses intended for slaughter were stallions. This is an important difference when compared to a study of slaughter horses in Europe where up to 15% of horses transported for slaughter were stallions (Marlin *et al*., 2011). The percentage of stallions in the consignment could have a big impact on the way these horses are segregated and in turn affect the stocking density of the load. Stallions generally need to be segregated to reduce aggression related injuries.

Assuming that all of the data on dead-on-arrivals during 2009 were included within the information supplied by the USDA, the number of dead-on-arrival horses during

2009, was low (2 horses) in comparison to the 2001 to 2006 data presented by the Alberta Equine Welfare Group (Alberta Farm Animal Care, 2008).

In conclusion, this study provided information to understand the journey characteristics and animal characteristics of slaughter horses from the USA to Canadian slaughter plants. More detailed observations regarding origin of journey, time of unloading, rest periods if any, and standardized terminologies for the owners to self-declare fitness issues before transport are some of the improvements needed in the owner/shipper certificate forms. It also provided useful information to place the results from subsequent detailed studies of specific journeys to one Canadian slaughter plant (chapter 6) into context.

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CHAPTER 3.

WELFARE ISSUES ASSOCIATED WITH THE TRANSPORT AND SLAUGHTER OF HORSES IN ICELAND

3.1. INTRODUCTION

Despite the increased mechanization and roadway improvements that diminish the necessity for their use, Icelandic horses still play a significant part in daily life in Iceland. Farmers still use horses to round up sheep in the Icelandic highlands, and they are also used for pleasure riding and in special gait competitions. Apart from these uses, they are farmed for meat purposes. The population of horses in Iceland has increased steadily from 30,000 in 1961 to 80,000 in 2009 (FAO statistical database, 2010). The Farmers Association of Iceland reported that in 2006 there were 2,389 registered horse breeding farmers with an average herd size of 23 horses (The Farmers Association of Iceland, 2009). This data accounts for 69% (54,947 horses) of the total horse population and the remaining 31% may be riding horses raised in stables by urban dwellers.

Even though the horse population in Iceland has risen since 1960, the number of horses slaughtered for meat has not increased (Figure 3.1). During the period from 1961 to 2009, the number of horses slaughtered each year in Iceland for meat has remained relatively stable – between 7,000 to 8,000 horses (FAO statistical database, 2010). Horse meat consumption in Iceland is marginal (Lombardi-Boccia *et al.*, 2005). Of the total meat production (lamb, poultry, pork, beef, and horse) in Iceland, horse meat production comprises 2.6% (The Farmers Association of Iceland, 2009). According to the Farmers

Association of Iceland's data, horse meat consumption in Iceland decreased from 3.2 kg of horse meat per capita in 1983 to 1.7 kg per capita in 2010. However, the same data also indicated that there was a steady increase in the export of horse meat to other countries, such as Finland and Switzerland, (0 tonnes to 312 tonnes) during this same period which compensated for the decline in domestic horsemeat consumption.

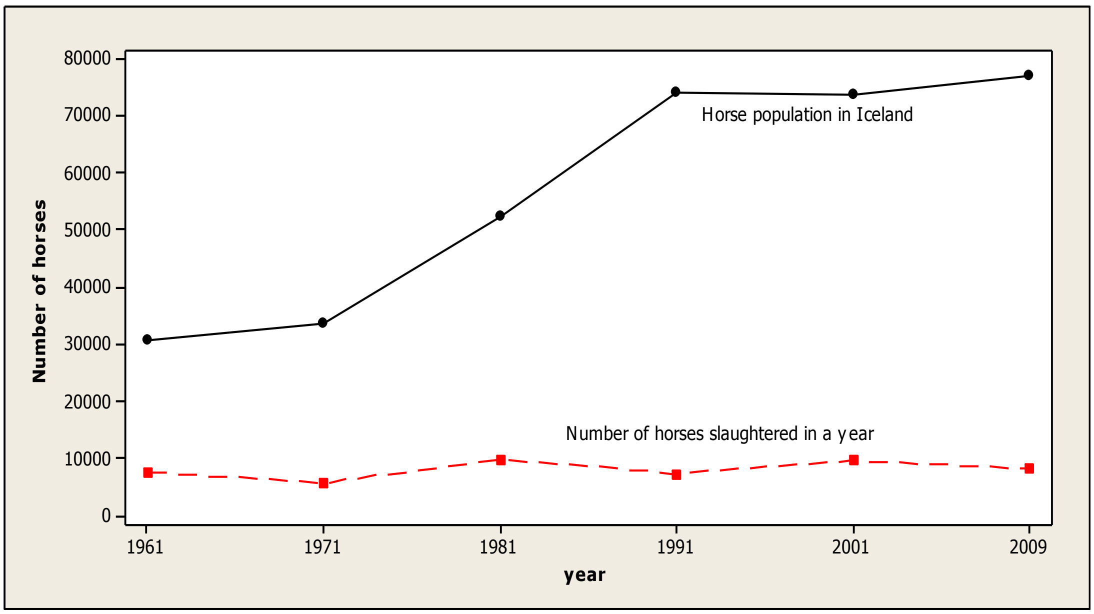

Figure 3.1: Number of horses slaughtered compared to horse population per year in Iceland during the period from 1961 to 2009 according to the FAO statistical database. Bold black line shows the steady increase in horse population, and dotted red line shows the relatively stable number of horses slaughtered every year.

Some of the factors that may have contributed to this trend of increased consumption of horse meat in some European countries could be tenderness of foal meat (Franco *et al.*, 2011), excellent nutritional properties (Badiani *et al.*, 1997) and food safety problems in other livestock products, such as Bovine Spongiform Encephalopathy (BSE), dioxins and swine influenza (Pickelsier and Wahl, 2002). It is interesting to take note here that the recent horse meat scandal in Europe which involved adulteration of non-horse meat products with horse meat has increased interest in the slaughter of horses for human consumption.

The climatic conditions in Iceland during winter are harsh with seven month of snow cover from November to May (Einarsson, 1984). In this climate, livestock production such as cattle and sheep farming are difficult to maintain under free range conditions. However, unlike other livestock, Icelandic horses seem to thrive under harsh snowbound winter conditions without additional feed supplementation (Gudmundsson and Dyrmundsson, 1994).

Icelandic horses are regarded as robust and long lasting (Björnsdóttir *et al.*, 2003) with a double coat of hair for extra insulation in cold temperatures. Evidence from previous studies has shown that growth rate of Icelandic horses compared favourably with those of other livestock species, even when they were grazed on poor-quality pastures (Gudmundsson and Dyrmundsson, 1994). The production of horse meat provides a high dressing percentage, closer to 60–70% in meat yield which is higher than that of other meat producing livestock (Lanza *et al.*, 2009), which offers added economic value.

Icelandic horse breeders calculate how many horses they can feed with the poor vegetation in the pasture during winter and control the stock size accordingly (Schwanke, 2009). Every year in autumn, the farmer downsizes the herd by culling surplus horses, which are then sent for slaughter in preparation for the winter ahead. Thus, a farmer may decide to send some foals (6 months to one-year-old) for slaughter along with a few adult mares.

In recent times the welfare of horses during transport for slaughter has been of significant worldwide public interest. However, there is an absence of information regarding the state of the horses on arrival at the slaughter plant after transport and the methods of handling of horses during and after transport in Iceland. Research conducted in other European countries has reported a high prevalence of injuries during transport among slaughter horses (Marlin *et al.*, 2011). Research conducted in the United States of America (USA) has indicated that dehydration, fatigue, and injuries were major welfare issues among horses transported for slaughter (Grandin *et al.*, 1999; Stull, 1999). Experimental work which mimicked the commercial transport of horses for slaughter by not providing access to water during hot conditions, found signs of dehydration and fatigue after a journey duration of 28 hours (Friend, 2000; Friend, 2001). In Iceland, the transport conditions, and journey durations are different from those in North America. The journey durations are short, the terrain mountainous and the climatic conditions are also different. The reason for a short duration of transport is because geographically, Iceland is a small country, and slaughter plants are spread across the country.

The slaughter procedure for horses involves standard procedures such as restraint, stunning, hoisting, and exsanguination. The pre-slaughter procedures can be stressful if adequate precautions are not taken. Welfare problems have been previously identified in Chile regarding the slaughter procedure of horses (Werner and Gallo, 2008). These authors reported that each horse needed an average time of 9 minutes in the stunning box to be stunned. Forty three percent of horses showed behavioural signs of return to consciousness after stunning and 14% required a second captive bolt shot for stunning. These results indicate that the horses slaughtered in Chile were likely to have experienced potential welfare issues, such as pain and distress. Grandin (2010) suggested that a good standard for slaughter plants to follow is that only one percent of the animals should require to be stunned a second time. There is no published data available on the slaughter procedure or stunning efficiency of slaughter horses in Iceland.

The objectives of this study were to:

1. Quantify journey characteristics of horses transported for slaughter.

2. Assess the welfare of horses before and after transport to a slaughter plant.

3. Assess equine welfare during transport and slaughter (through examination of carcass and blood parameters).

4. Examine relationships among journey characteristics and welfare assessments.

5. Assess stunning efficiency.

3.2. MATERIALS AND METHODS

3.2.1. Sampling plan

Over a ten-week period, in November and December of 2010 and 2011, 46 truckloads of horses transported for slaughter in Iceland were studied at one of the biggest slaughter plants in Iceland. All truck loads of horses transported during the study period were sampled. During the loading process, one to eleven horses were chosen by a convenience sampling method from each truckload by noting down the colour and markings of the horse. Particular importance was given to include adult horses during the sample selection, because fewer adult horses were transported than foals. One hundred and eighty-eight horses were selected (from 910 total horses) for detailed study from the 46 truckloads of horses transported. Of the 188 horses observed, 59 were adults and 129 were foals.

3.2.2. Vehicle used for transportation

Figure 3.2: Non-articulated single deck livestock vehicle used for the transport of horses. It has three equal sized compartments inside.

The majority of horses slaughtered in this plant were transported by one non-articulated livestock vehicle (Figure 3.2) which belonged to a contracted transporter. The animal holding area of this vehicle had 17.7 m^2 of space, divided into three equal parts. The vehicle had a hydraulic tail gate lift in the rear end, which was used for loading the horses into the vehicle. The temperature inside the vehicle was recorded every 3 minutes from 14 consignments using a data logger (Tiny tag® temperature recording thermometer, Gemini Data loggers (UK) Ltd, Scientific House, Terminus Road, Chichester, West Sussex, PO19 8UJ, England) attached to the ceiling of the middle compartment. Before the horses were loaded into the truck, the external air temperature and humidity were measured using laser thermometer (Mastercraft Hawk eye-Canadian Tire) and hygrometer (Magna-Temp® *RH*% meter) respectively.

3.2.3. Management of horses for transport and slaughter

The day before or on the day of transport to the slaughter plant, horses were driven from the grazing pasture to the holding area which consisted of a wooden enclosure located near a road (Figure 3.3). This holding area led to a race, from which the horses could be loaded into the vehicle. Horses were driven from the wooden enclosure into the hydraulic lift and loaded into the three compartments. Adult horses and foals were transported together in the vehicle.

Figure 3.3: Horses rounded up into an enclosure before transportation to the slaughter plant which was normally built near the roadway of every farm.

Figure 3.4: Foals stabled in the lairage pen as groups. One mare (visible on the left-hand side) had been separated from its foal.

The number of horses that were loaded into the three compartments of the vehicle was dependant on the total number of horses transported, which varied from trip to trip. Due to this variability and difficulty in observing the number of horses loaded into each compartment on each load, stocking density and space allowance could not be calculated precisely at the compartment level. However, as the total number of horses in each load was observed consistently, stocking density was estimated at the load level by assuming an equal distribution of horses among compartments.

Unloading of horses at the slaughter plant after transport was performed via a ramp which led directly to the lairage. However, after unloading from the vehicle; adults and foals were separated in the lairage and this also acted as the separation or weaning procedure for the foals (Figure 3.4).The horses were held overnight. The time interval between unloading after transport and slaughter ranged from 11 to 19 hours. The stocking density in the lairage was high and space available for a researcher to undertake any detailed measurement regarding stocking density was restricted. Hence, the group size of the horses in the lairage (calculated from the number of horses transported for slaughter on that day) was recorded and stocking density in the lairage was categorized as low (<80 horses), moderate (80 to 100 horses) and high (>100 horses). No feeding was carried out during lairage. Water was provided through automatic waterers (nipple drinkers). The next morning, horses were driven towards the stunning pen from the lairage pens by the person who subsequently stunned them. Stunning was performed using a captive bolt pistol followed by sticking (exsanguination).

3.2.4. Recording protocol

Journey characteristics, such as the source of the truck load, starting time of the journey (when the first horse went inside the truck), finishing time (when the last horse left the truck), and details on the number of horses loaded on to each vehicle were recorded. Observations were started when the transport vehicle reached the farm and the loading of horses began. The horses were assessed for the presence of any pre-existing health conditions such as wounds, lameness, and deformity during loading. Skin temperature, respiration rate, and other health variables such as general attitude (alert, apathetic, severely depressed and agitated/excited), body condition score (scale of 1 to 5) (Burn *et al.,* 2010), lameness, injuries (wounds), and presence of sweating (wetness of skin) were observed in the 188 selected horses immediately before loading and after unloading. Skin temperature was measured with an infrared thermometer (TPI 381® Non-Contact Thermometer, Test products International, Ltd. 342 Bronte St. South, Unit 9, Milton, Ontario L9T 5B7) held at approximately 0.2 metres from the forehead region in between the eyes of the horse. Respiration rate was recorded by observing abdominal or nostril movements. Lameness was assessed using the following categories: no detectable abnormality (0), weight bearing on all limbs but noticeable defect in walking (1), weight bearing on all limbs while walking but visible or evident difficulty in walking (2), able to stand up and walk but foot raised so that the toe is touching ground while standing (3), non-weight bearing lameness (4) and recumbent (5).

The character, severity and size of wounds were assessed. Character was classified as the following: non bleeding open wound, bleeding wound, fibrin or dried exudates, infection (presence of pus), granulation wound and scar. Severity of the wound was assessed by the layers of skin involved (wound depth). Wound depth was described by the layer of tissue penetration as epidermal, dermal, subcutaneous, into muscle, into tendon, or into bone (Hollander *et al.*, 1995). The size of wound was categorised as:

- small (≤1 cm^2 or 1x1 cm or diameter 1.1 cm)
- medium (1.1 to 10 cm^2 or 3.3x3.3 cm or 1x10cm or 3.5 cm diameter)
- large (10.1-20 cm^2 or 4.5 x 4.5 cm or 2 x 10 cm or 3 x 6.5 cm or diameter 5 cm)
- very large (20.1-100 cm^2 or 10 x 10 cm or 2 x 50 cm or 3 x 33 cm or 4 x 25 cm or diameter 11 cm) and
- extensive (>100 cm^2).

The stunning procedure of selected horses (the same 188 horses) was also observed to identify behaviours which may indicate insufficient stunning, and the number of captive bolt shots needed for stunning was recorded. The slaughter procedure was evaluated using the criteria described by Grandin (1998). Rhythmic respiration, eye movements, vocalization, head elevation and attempts to stand were observed to determine whether the horse was conscious (sensible) when on the bleeding rail. The percentage of horses stunned with one captive bolt and the percentage of horses conscious on the bleeding rail were noted (Grandin, 1998).

During exsanguination, blood was collected from the selected horses using a plastic beaker, and a sample transferred to a vacutainer containing anti-coagulant lithium heparin. Within 2 minutes of blood collection, a drop of blood was removed from the beaker and the blood lactate concentration was measured using a lactate measuring meter (Lactate Pro LT-1710®, Arkray Inc, 57 Nishi Aketa-cho, Higashi-Kuji, Minami-ku, Kyoto, Japan) which had been previously validated for veterinary use (Thorneloe *et al.*, 2007). A second drop of blood was used to measure blood glucose concentration using an blood glucose monitoring meter (Alpha TRACK®, Abbot Laboratories, Borth Chicago, Illinois, 60064, USA) which had previously been validated for use in horses (Hackett and McCue, 2010). After centrifuging the blood for 20 minutes at 3000 rpm using centrifuge (SIGMA 1-15, D-37520 Osterodeam Hertz, Germany), plasma total protein concentration was measured using a temperature compensated hand-held refractometer (Reichert®, Reichert, Inc. 3362 Walden Avenue, Depew, NY 14043 USA). Packed cell volume was measured using a haematocrit reader (Hawksley Micro-Haematocrit, Marlborough Road, Lancing Business Park, Lancing, Sussex, BN15 8TN) after centrifuging the micro-haematocrit tubes with whole blood.

Post-mortem examination during meat inspection was undertaken to quantify bruising. Bruising was assessed using a subjective scoring system based on the Australian Carcass Bruise Scoring System (Anderson and Horder, 1979). The size of the bruise was categorised similarly to that of wound classification.

3.2.5. Statistical analyses

The average number of horses slaughtered annually in Iceland is approximately 8000. A sample size of 188 horses represents 2.4% of the population of horses slaughtered. Furthermore, to determine an appropriate sample size, a pilot study involving observation of 13 truckloads was undertaken and sample size estimation was made using that data. The only significant welfare issue identified from the pilot data was bruising, and hence, the sample size calculation was made with bruising as the outcome variable and journey duration as the predictor. It was hypothesized that the proportions of number of horses bruised at short journey durations (<100 minutes) and long journey durations (>100 minutes) are 0.2 and 0.4 respectively. Hence, at the power of 0.8 and alpha 0.05, the sample size required was 182 animals.

Respiration rate and skin temperature were not normally distributed (evaluated by histogram and the Shapiro-Wilk W test for normality) and hence comparisons before and after transport were undertaken using a Wilcoxon signed rank test. Comparisons of blood lactate concentration, blood glucose concentration, plasma total protein concentration and packed cell volume between adult and foals were made using two-sample t-tests (after assessment for normality). Occurrence of bruising was compared between adults and foals by cross tabulation and a chi-square test on the numbers of horses bruised.

Simple regression analyses were performed between the "difference in skin temperature before and after transport" (outcome) and duration of journey, stocking density on the vehicle, stocking density in lairage, vehicle temperature and external air temperature as predictors. This was undertaken to understand the factors which could cause thermal stress in horses during transport.

After examining descriptive statistics of the welfare outcomes, it was evident that three welfare outcomes were of potential interest, namely the prevalence of bruising, signs of dehydration indicated by increased plasma protein concentration and muscular activity indicated by high blood lactate concentration. Regression models were built for these three outcomes. For each welfare outcome, a causal diagram was drawn to illustrate biologically feasible risk factors from those recorded in the study and with sufficient variation. Unconditional associations between each risk factor and welfare outcome were evaluated by simple linear regression. All risk factors which showed significant unconditional association with the outcome variable were included in each model. Even though some risk factors did not have a significant effect on the outcome, they did change the coefficient in the conditional model. Therefore, all variables were included in all the three models.

The relationship between the outcome variable – bruising and risk factors were studied by building logistic regression model. Before building model unconditional associations between these two outcomes and predictors were evaluated. For the outcome bruising (presence or absence of bruising in the carcass), multivariable logistic regression analysis was performed. The predictor variables used were stocking density in the vehicle, age (adult or foal), journey duration and stocking density in the lairage. The random effect due to truckload was controlled by using a mixed model approach (using truckload as a random effect). However, truckload accounted for negligible unexplained variation in the final model and hence was dropped. Analytical evaluation for confounding and interaction was performed. The fit of the logistic model for bruising was evaluated using Hosmer-Lemeshow goodness-of-fit test. Specific observations not fitting the model or having undue influence on the model were evaluated by generating Pearson and deviance residuals and any outlying values examined.

The relationship between the outcome variable blood lactate concentration and risk factors were studied by building a linear mixed model (truckload as random effect). The predictor variables used were stocking density in the vehicle, age (adult or foal), journey duration and stocking density in the lairage. Truckload accounted for negligible unexplained variation on the final model and hence random effect was dropped. Analytical evaluation for confounding and interaction was performed. Fit of the model was performed by evaluation of heteroscedasticity and normality of the residuals. Stata 12 (StataCorp, 4905 Lakeway Drive College Station, Texas 77845 USA) was used to perform all statistical models.

3.3. RESULTS

3.3.1. Journey characteristics

Table 3.1 lists descriptive statistics for the risk factors studied which could have had an effect on the welfare of the horses transported. Journey duration to the slaughter plant ranged from 20 minutes to 185 minutes. The number of horses loaded in a truck (in all three compartments combined) ranged from 7 to 35. The space allowance provided per horse ranged from 0.4 to 1.9 m^2 per horse (assuming equal distribution between compartments). Stocking density ranged from 117 to 460 kg/m^2 which was calculated from the extrapolated live weight, as only carcass weight measurements could be obtained. As previous studies have shown that average carcass yield of horses is approximately 60% (Lanza *et al.*, 2009; Sarriés and Beriain, 2005), live weight was extrapolated from the carcass weight.

Table 3.1: Descriptive statistics for journey and environmental conditions that could potentially affect the welfare of horses transported for slaughter in Iceland.

Risk factors studied	**Units**	**n**	**Min**	**Q1**	**Median**	**Q3**	**Max**
Journey duration	minutes	46	20	60	71	120	185
No. of horses in the vehicle		46	7	15	20	25	35
Adults		46	0	0	2	4	7
Foals		46	0	15	17	22	34
Stocking density in vehicle	kg/m^2	46	117	144	182	204	460
Space allowance in vehicle	m^2/horse	46	0.40	0.84	1.13	1.41	1.97
External air temperature	º C	46	-9	-3.5	-1	4	10
External humidity	%	46	25	40	53	58	90
Air temperature in vehicle†	ºC	12	0	7	9	11.7	16.4

Q_{1}=first quartile & Q_{3}=third quartile

†Maximum temperature recorded every three minutes by the data logger

3.3.2. Health of horses before and after transport

Table 3.2 lists the descriptive statistics for respiration rate and skin temperature measured before and after transport. Horses showed a significantly increased respiration rate and skin temperature after transport compared to before transport values (P=0.02). Foals had a significantly higher respiration rate than adults (P=0.02). However, no significant differences in skin temperatures were found between adults and foals.

Table 3.2: Comparisons of respiration rate and skin temperature, before and after transport for Icelandic adult horses (n=59) and foals (n=129).

Parameter	Units	Category	Before transport					After transport				
			Min	Q_1	Median	Q_3	Max	Min	Q_1	Median	Q_3	Max
Respiration rate	Breaths/ min	Adult	24	30	32	40	41	28	37	43	50	68
		Foal	24	35	40	55	61	28	44	55	78	95
Skin temperature	ºC	Adult	0.5	1.7	4.2	8.9	15.6	1.1	4.4	11.4	13.3	22.2
		Foal	0	1.7	3.3	9.4	12.8	1.1	5.5	11.1	14.4	22.2

Three horses (point prevalence of 1.6%) had visible wounds after transport, whereas no visible wounds were observed before transport. All three horses had small sized (1 × 1 cm), bleeding and superficial wounds in the hock region. Body condition of all horse transported were equal to or more than 3 (in the body condition scale of 5), and none of the horses (adult and foals) observed had any pre-existing condition or lameness. Overall 3% of horses (adults and foals combined) were apathetic after transport while none were apathetic before transport. Thirteen % of horses had a wet coat after transport.

Table 3.3 and Figure 3.5 illustrate the percentage of horses which had abnormal physiological values when compared to the normal clinical range. Blood lactate concentration was highly elevated in 100% of adults (mean value of 5.35 mmol/litre) and foals (mean value of 6.1mmol/litre) when compared to the normal clinical range (1.1-1.7mmol/litre) for horses (Kaneko *et al.*, 1997). Foals had significantly higher blood lactate concentration than adults (P=0.002). Seventy-eight % of adults and 58 % of foals had a blood glucose concentration within the clinical range (4.1-6.3 mmol/litre). Eighty % of adults and 93% of foals had a packed cell volume within the normal clinical range (32-53%). Total plasma protein concentration was in the normal clinical range for 71 percent of foals; however, 58% of the adults had higher than the normal clinical range.

Table 3.3: Percentage of adult and foal Icelandic horses with normal and abnormal blood variables measured at exsanguination.

	Normal clinical range[1]	Age	n	% of horses		
				Below range	Within range	Above range
Blood lactate concentration	1.1-1.7 mmol/l	Adult	59	0	0	100
		Foal	126	0	0	100
Blood glucose concentration	4.1-6.3 mmol/l	Adult	59	13	78	8
		Foal	126	20	58	22
Total plasma protein concentration	52-79 g/l	Adult	49	2	40	58
		Foal	121	4	71	25
PCV	32-53 %	Adult	49	2	80	18
		Foal	111	0	93	7

[1] Kaneko *et al*. (1997)

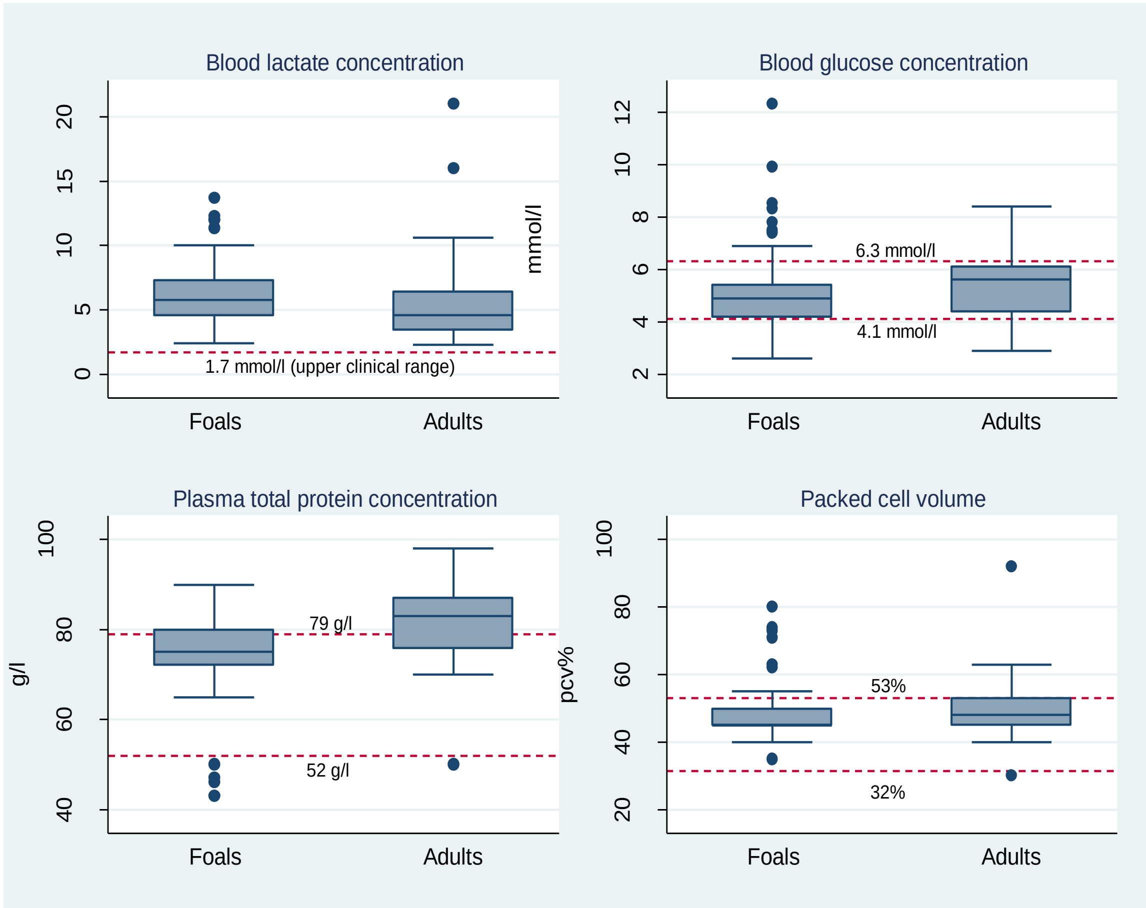

Figure 3.5: Box plot showing the blood concentration of lactate and glucose, plasma concentration of total protein and packed cell volume of foals and adults in Icelandic slaughter horses. The boxes indicate the upper and lower quartiles with the median as the line in-between. The whiskers indicate the maximum and minimum values. • indicates an outlier, defined as those values which are 2.5 time the interquartile range from the median value. The dashed red horizontal lines indicate the clinically normal range.

Among the 57 adult carcasses observed, 25 had bruises (prevalence of 44%) and among the 127 foal carcasses observed, 22 had bruises (prevalence of 17%). All bruises were in the medium-size category.

3.3.3. Associations between journey characteristics and skin temperature

When unconditional associations were studied by simple regression analysis with each truck load as a random effect; journey duration, maximum vehicle temperature recorded during the journey and external air temperature recorded before loading of horses were the three factors which had a significant effect on the skin temperature difference before and after transport (Table 3.4). Stocking density did not have a significant effect on the increase in skin temperature.

Table 3.4: Unconditional simple regression analysis between difference in skin temperature before and after transport (outcome) and predictors: journey duration, stocking density in the vehicle, maximum temperature recorded inside vehicle during transport, and environmental temperature. Variation between truck loads was built as a random effect in the regression models.

Predictor	No. of truckloads	Coefficient (º C)	P value	Confidence interval
Journey duration	40	0.020	0.017	0.004 to 0.036
Stocking density in the vehicle	23	0.001	0.786	-0.003 to 0.004
Maximum vehicle temperature	14	0.297	0.035	0.0208 to 0.572
Environmental temperature	40	0.156	0.020	0.024 to 0.288

3.3.4. Associations between welfare outcomes and risk factors

When unconditional associations were studied for the occurrence of bruising, the risk factors; age (adult or foal) and stocking density in the truck were identified as factors that were significantly associated with bruising. However, when a multivariable model was built with prevalence of bruising as the outcome and age, stocking density in the vehicle, duration of transport and stocking density in the lairage, the only factor that significantly affected the occurrence of bruising was age (adult or foal) (Table 3.5).

Table 3.5: Odds ratio, standard error (SE), significance, and confidence interval of a logistic regression model to determine risk factors that could influence the occurrence of bruising measured in horse carcasses.

Risk factor		**Odds ratio**	**P Value**	**Confidence interval**
Journey duration		1.00	0.8	0.99 - 1.01
Stocking density in the truck		0.98	0.08	0.96 - 0.99
Age	Foal	Ref	Ref	Ref
	Adult	3.67	0.006	1.45 - 9.22
Stocking density in the lairage				
• Low		Ref	0.78†	Ref
• Moderate		1.47	0.55	0.40 - 5.33
• High		1.54	0.74	0.17 - 5.53
Constant		1.42	0.74	0.17 - 11.53

†Overall P value for categorical variable, Ref = reference level

When the unconditional association of blood lactate concentration with risk factors was studied, the following predictors had a significant effect: age (adult or foal), stocking density in the lairage and environmental temperature. However, when a model was built, age (adult or foal) and stocking density in the lairage were the significant factors affecting the blood lactate concentration (Table 3.6). The median blood lactate concentration in horses kept at the high lairage stocking density lairage category was 4.7 mmol/l (Q_1=2.8, Q_3=6.1), at the medium category it was 4.2 mmol/l (Q_1=2.3, Q_3=5.6) and at the low category it was 3.3 mmol/l (Q_1=2.3, Q_3=4.6). When a model was built for plasma total protein concentration, only age (adult or foal) had a significant effect, adults having significantly higher total plasma protein concentration than foals (Table 3.7)

Table 3.6: Multivariable linear regression of risk factors affecting blood lactate concentration (log transformed) measured from blood collected during exsanguination.

Risk factor	**Coefficient**	**P value**	**Confidence interval**
Journey duration	0.001	0.25	-0.0006 to 0.0023
Age			
Foal	Ref	Ref	Ref
Adult	-0.244	0.001	-0.3918 to -0.096
Estimated stocking density in vehicle	-0.001	0.37	-0.0016 to 0.0011
Stocking density in lairage			
• Low	Ref	0.04†	Ref
• Moderate	0.203	0.03	-0.0107 to 0.395
• High	0.221	0.02	-0.0280 to 0.414
Constant	1.541	0.001	1.286 to 1.796

†Overall P value for categorical variable, Ref=Reference level

Table 3.7: Multivariable linear regression of risk factors affecting plasma total protein concentration measured in blood collected during exsanguination

Risk factor	Coefficient	P value	Confidence intervals
Journey duration	-0.02	0.51	-0.066,0.033
Estimated stocking density in vehicle	-0.03	0.05	-0.06,-0.01
Age			
Foal	Reference	Reference	Reference
Adult	7.28	<0.01	4.42,10.14
Estimated stocking density in lairage			
Low	Reference	Reference	Reference
Medium	-0.84	0.79	-7.09,5.41
High	3.88	0.21	-2.23,9.98
Constant	79.82	<0.001	73.05,86.59

[1]Overall P value for categorical variable

3.3.5. Assessment of welfare at stunning

Out of 188 horses observed, two horses were given a second shot of the captive bolt for stunning because the first shot was ineffective. Three other horses showed signs of insufficient stunning that showed either signs of rhythmic breathing or head elevation behaviour after stunning and hoisting (stunning efficiency of 98.4 %).

3.4. DISCUSSION

In Iceland, even though journey duration was short, the presence of visible wounds after transport when compared to before transport and the presence of bruising after slaughter indicated that there are welfare issues associated with the transport and slaughter of horses. Prevalence and severity of wounds in Icelandic slaughter horses

(1.6% minor wounds) was lower than that seen in slaughter horses in European countries (28% acute injury) (Marlin *et al.*, 2011). In the USA, 2% deep wounds were reported by Grandin *et al.*, (1999).

Prevalence of bruising in Icelandic slaughter horses was higher, particularly in adult horses (44%), when compared to studies in USA slaughter horses (25%) (Grandin *et al.*, 1999). One plausible reason for the high percentage of bruising observed in adult horses may be associated with blunt trauma due to handling and not due to transport associated blunt injuries as observed in Canada (Chapter 6). Grandin *et al*., (1999) identified that 19% of slaughter horses that arrived at a slaughter plant in the USA had a pre-existing condition or injury that made them unfit for transport. Eight per cent of the horses that arrived at the slaughter plant were classified as having a severe welfare problem, 6% due to conditions that originated before transport and 2% due to injuries that occurred during transport and marketing.

Some causative factors which have been identified by previous studies associated with injuries during the transport of horses are kicking and biting (Grandin *et al.*, 1998), high stocking density (Iacono *et al.*, 2007; Tarrant *et al.*, 1988) and mixing of horses of different groups (Knubben *et al.*, 2008; Mach *et al.*, 2007). Aggressive behaviour such as kicking and biting were not observed during this study among Icelandic horses; however, a quantitative method of behavioural assessment for aggression related events (for example, video recording of behaviours) was not used in this study and hence there is a need for further investigation. Forceful handling by handlers such as pushing was observed anecdotally as a cause for foals falling down thereby receiving minor wounds, and adults horses being subjected to blunt trauma during unloading. Rough handling

(Costa *et al.*, 2006; Jarvis and Cockram, 1994) had been identified as a causative factor for injuries previously in the transport of slaughter cattle and sheep, but not in the transport of slaughter horses. The association between development of wounds and bruising with handling of horses such as pushing during loading and unloading by the transport staff needs to be examined further.

Risk analysis for bruising indicated that being an adult increased the odds of occurrence of bruising by 3.6 times compared to foals in this study. One plausible reason could be that adult horses when transported in groups are more likely to exhibit increased aggressive behaviours such as kicking and biting in comparison to foals. Young horses generally tend to be less aggressive than adults (Houpt *et al.*, 1978). Though the occurrence of bruising was significantly lower in foals than in adults, the observed prevalence of bruising in foal carcases is still a concern in terms of welfare. One possibility is that foals may be getting injured by adult mares during transport. In addition, there was mixing of foals from different farms, particularly in the lairage which could initiate aggressive behaviours, such as kicking and biting among foals.

Space allowance provided during transport was below the minimum standard for majority of consignments as per European recommendations for horses. The minimum recommended standard in the European Union Directives for adult horses and foals is 1.4 m^2 (Council of the European Union 2005). Seventy eight percent of consignments studied had space allowance provision below 1.4 m^2. Stocking density in the lairage was high as shown in Figure 3.4. The European Commission Directive 98/58 EC specifies ‘freedom of movement’ under all housing conditions including lairage. World organisation for Animal Health (OIE) guidelines also specify ‘appropriate species-specific shelter’ needs to be provided in slaughter plants. Interestingly, high stocking density in the lairage was significantly associated with the blood lactate concentration of the horses.

There was a significant increase in respiration rate after transport compared to before transport in adults and foals. This increase in respiration rate could potentially have been because of dehydration, stress, high metabolic activity or, excitement of being in a new environment. The normal respiration rate in healthy Icelandic horses is 23±2 breaths /minutes (Gehlen *et al.*, 2008). The respiration rate recorded before transport was 33±5 breath/minutes for adults and 42±11 breaths/minutes for foals. After transport, the respiration rate increased to 44±9 breaths/ minutes in adults and 58±11 breaths/ minute in foals. The increased respiration rate before transport (higher than the normal clinical range, i.e. 23 breaths/min) could be because some handling which had taken place to drive the horses into the collection area. The high respiration rate after transport could also be attributed to the stress associated with the transport.

Ambient temperature within the vehicle during transport and skin temperature of selected horses were measured before and after transport to evaluate whether any thermal stress was likely in horses transported in Iceland. Even though, the environmental temperature in Iceland was below 0º C most of the time (-9 to 10º C range during the study period), previous studies had established that -9ºC is not below the lower critical temperature of Icelandic horses (Autio *et al.*, 2007; Mejdell and Boe, 2005). The lower critical temperature is a limit value for the ambient temperature below which both an animal's metabolic rate and the rate of non-evaporative heat loss increase linearly. The thermo-neutral zone of mature Quarter Horses, accustomed to mild winter temperatures is considered to range from approximately –15 °C to 10º C (McBride *et al.*, 1985).

Temperature recordings inside the vehicle during transport indicated that the temperature gradually increased from subzero temperatures to positive values as the duration of travel increased. However, the temperature inside the vehicle never exceeded 16.4º C in any of the loads studied. In addition, the skin temperature of the horses after transport ranged from 1 to 22 ºC. As the ambient temperature inside the vehicle was within the upper and lower critical range for horses and skin temperature of horses was within acceptable range, it appears that these horses did not suffer from thermal stress (extreme cold or heat). The skin temperature difference between before and after transport was significantly increased by journey duration and environmental temperature. Therefore, it is interesting to understand that thermal stress was minimized by the short journey duration in Iceland under current transport conditions and increasing the journey duration might increase the risk of thermal stress.

Lactate concentration indicative of anaerobic muscle metabolism can be increased after 12 h of a 24-h journey and remain elevated for 12 h after transport (Stull and Rodiek, 2002). In the current study, the median blood lactate concentrations at exsanguination were equivalent to those recorded after exercise and the maximum concentrations were similar to those obtained after vigorous exercise (Harris and Snow, 1992; Stefánsdóttir *et al.*, 2014). It is therefore, possible that muscle exercise during handling and transport could have raised the blood lactate concentrations. However, journey duration and estimated stocking density in the truck did not significantly influence blood lactate concentration. In addition, as blood lactate concentration in this study was measured in blood collected during exsanguination, it was difficult to attribute the raised blood lactate concentration to transportation alone. Stocking density during lairage significantly affected blood lactate concentration and the higher than normal values could also have been caused by the slaughter procedure. Werner and Gallo (2008) measured plasma lactate concentration before transport, after loading, after journeys of between 0.75 and 1.5 h, after unloading and after 18 to 21 h of lairage. Plasma lactate concentrations after transport and lairage were significantly raised compared with that before loading. However, they found that the greatest increase in plasma lactate concentration occurred between blood collected in the stunning pen and that collected during exsanguination, about 10 minutes after the stunning pen sample was collected. It is likely that vigorous muscle movement following captive bolt stunning and possibly catecholamine release (Werner and Gallo, 2008) were responsible, at least in part, for the raised blood lactate concentrations. Although in pigs, lactate concentrations measured in blood collected during exsanguination has been used to provide information on the effects of pre-slaughter handling (Edwards *et al.,* 2010); the interpretation of these results is complex. Although dehydration has the potential to affect blood lactate concentration, especially if the packed cell volume is raised, only 18% of the adult horses had a packed cell volume that might have affected blood lactate measurement (Evans and Golland, 1996). The greater blood lactate concentrations in foals than in adults showed that age affected the response to handling, transport, lairage and/or slaughter.

Blood glucose concentration was measured to evaluate the effects of food deprivation. In this study, 78.5% of adults and 58% of foals had blood glucose concentrations, which were within the normal clinical range. Around 10% of adults had lower concentration and another 10 % had higher concentration. Similarly, in foals, approximately 20% of foals had higher concentration and another 20% had lower concentrations. Changes in blood glucose concentration can be influenced by feeding patterns (Sticker *et al.*, 1995), exercise patterns (Hyyppä, 2005) and also in response to the activation of the adrenal axis. Horses transported for a short distance (1 hour) can show a significant increase in blood glucose concentration (Werner and Gallo, 2008). It is obvious, that in such a short duration of transport, an effect of food deprivation on blood glucose will be negligible. Therefore, this increase in blood glucose could be attributed to a stress response due to release of adrenaline and cortisol. Horses transported on long journeys (24 hours) with or without feeding provisions also showed an increase in serum glucose (Stull and Rodiek, 2002) and plasma cortisol concentration (Friend, 2000). Comparisons between transport (different journey durations) and exercise regimens on blood glucose concentration are also an effective way to interpret the physiology responses. Plasma glucose concentration tends to decrease during prolonged exercise (>3 hours) (Rose *et al.,* 2008). However, during short intense exercise both decreases and increases have been recorded (Snow and Mackenzie, 1977; Lindholm and Stalin, 1974). The blood glucose concentration was within normal range for most of the horses. The raised blood glucose concentration in some of the horses may have been associated with exercise or excitement and this was unlikely to have been associated with any welfare issues. The lower than normal blood glucose concentrations in 20% of the foals and 13% of the adults might have been reflective of a period of food deprivation. However, the low blood glucose concentrations were well above those associated with clinical significance.

The normal clinical range of total plasma protein concentration recorded in Icelandic horses reported by Unkel (1984) was 61.5-74.5 g/l, but the higher limit was lower than the clinical range indicated by Kaneko (1997). After transport, adults showed a mean total protein concentration of 81.5 g/l and foals 74 g/l. Fifty-seven % of the adults had a higher total plasma protein concentration than the higher limit of clinical range indicated by Kaneko (1997). However, only 25 % of foals had higher than normal total protein concentration. The high percentage of adults showing elevated total protein concentration could be due to most adult horses being mares, which had been lactating making them more prone to dehydration. However, previous studies have shown that when there is normal feeding and watering management, pregnancy or lactation does not have a significant effect on total plasma protein concentration (Harvey *et al.*, 2005). Under Icelandic conditions, intake of water during transport and in lairage were minimal because there was no provisions for watering before or during transport and water was not readily accessible in lairage as horses may not have been accustomed to nipple drinkers which was used in lairage. The adult mares which had been lactating may have been at greater risk of dehydration, because of the additional water requirements of lactation (Harris, 2003; Merck & Co., 2008). However, the periods without access to sufficient water might not have had a severe welfare impact as feral horses drink only once in 24 hours, unless the weather is hot and humid (Feist and McCullough, 1975).

Loading and unloading conditions are generally considered stressful for horses (Shanahan, 2003; Waran *et al.*, 2002). In this case, a hydraulic tail gate lift system was used for loading the horses, and a permanent level ramp was used for unloading. In pigs, very little difference in stress levels has been found during loading and unloading when either a ramp or a hydraulic tailgate lift is used (Brown *et al.*, 2005). However, there is no scientific information available for horses regarding stress levels in response to loading using hydraulic lift systems. Apart from usage of the hydraulic lift, there was considerable handling involved while loading and unloading which could be a risk factor for injuries and hence needs to be studied in detail.

Rifle shot and captive bolt shot have been used by different slaughter plants for stunning of horses. In this slaughter plant, the captive bolt method of stunning was used. Only two horses received a second captive bolt shot for stunning out of the 188 observed. One study from Chile reported that 14% of horses were given a second captive bolt shot for stunning (Werner and Gallo, 2008). Captive bolt stunning can have good stunning efficiency if performed properly, particularly on small breeds of horses such as Icelandic horses. The number of captive bolt shots needed for stunning may not give an accurate indication of stunning efficiency because if the stunner stops after one captive bolt without giving much importance to check whether the animal is unconscious or not, the animal will suffer. In contrast if the operator is 'over diligent' they might give 'unnecessary' second shots to make certain that the horse had been stunned correctly.

Observation of behaviour for stunning efficiency (animal-based assessment) indicated a stunning efficiency of 98.4%. Grandin (2010) indicates that a good stunning practice for a slaughter plant was to have a minimum standard of 99% horses stunned by one shot (Grandin, 2010). As per Grandin (2010) standards, this Iceland slaughter plant has reasonable welfare standards in terms of stunning. One questionable practice observed during stunning was the accommodation of two horses in the stunning box simultaneously in order to reduce the work load for the person involved in stunning. This could be a serious welfare problem because both animals were not stunned at the same time. While the second animal was stunned, the first one was down in the narrow stunning box waiting for exsanguination. Another recommendation is that to have a captive bolt ready in the sticking (exsanguination) area, should a second shot be needed.

In conclusion, although there were no major injuries or physiological disruptions observed, there were some welfare issues identified by this study, particularly bruising associated with transport and slaughter-related activity. Handling, high stocking density during transport and in lairage mixing of adult and foals during transport and mixing of foals from different farms in the lairage could be risk factors associated with bruising. The observation that there is potential for dehydration needs to be addressed by correcting the water delivery method. Issues which need further investigation are impact of handling procedures on horses during transport and lairage and behaviour observation of horses to identify risk factors for bruising.

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CHAPTER 4

FACTORS AFFECTING THE MEASUREMENT OF SKIN TEMPERATURE OF HORSES USING DIGITAL INFRARED THERMOGRAPHY

4.1. INTRODUCTION

Skin temperature as measured by digital infrared thermography (DT) may be used effectively for diagnostic purposes when all the factors which affect the skin temperature are quantified. Energy emitted through the skin of an animal, measured as skin temperature varies depending upon surface temperature, surrounding environmental factors, and the surface characteristics being imaged (Holst, 2000). Outdoor factors such as sunlight, humidity, ambient temperature (Alsaaod and Büscher, 2012) and wind speed (Westermann *et al.*, 2013) may also have an effect on skin temperature measured. However, Eddy *et al* (2001) argued that as DT measures the heat emitted from the surface of the skin, it may not be affected by changes in intensity of light in the environment. The technique used by the operator may also affect outcomes including distance from the target, viewing angle and motion. Factors associated with the animal itself and its individual response to the surrounding environment may also influence the qualitative or quantitative data generated by DT. These include the level of activity prior to assessment (Gloster *et al.*, 2011), the physiological state of the animal (e.g. pregnancy) (Autio *et al.*, 2006), the length and density of the hair coat (Tunley and Henson, 2004), any concurrent pain or stress (Stewart *et al.*, 2008a; Stewart *et al.*, 2008b), and disease status (Bowers *et al.*, 2009). Endogenous animal factors may be difficult to compensate for in DT data analysis, but it is hypothesized that the techniques can be standardized, if environmental factors are understood and accounted for in the interpretation of results.

In human medicine, thermography is often used as a diagnostic tool under controlled environmental conditions (Love, 1980). However, in veterinary medicine, it is often difficult to control the environment in which thermographic images are taken (Bowers *et al.*, 2009; Gloster *et al.*, 2011). Some of the published work reporting on the use of DT with horses under outdoor conditions suggests that it may be used successfully in such circumstances. One group of researchers used DT on horses to examine cold tolerance in different seasons in a riding arena (Autio *et al.*, 2006). However, in a subsequent study, problems were reported in accurately determining heat loss patterns with DT at very cold temperatures (-21^{0} C), particularly when snow was present on the animal's body surface (Autio *et al.*, 2007). Another study which examined the reliability and repeatability of skin temperatures measured by DT found that the skin temperature patterns in horses were reproducible for up to seven days when environmental conditions were similar, suggesting that there may not be a need for an equilibration time (Tunley and Henson, 2004).

The World Organization for Animal Health (OIE), various legislative bodies, and industry organizations have welfare standards for the slaughter, transport, and killing of animals for disease control (OIE 2012a; OIE 2012b; Grandin, 2010). Among these standards are criteria for the assessment of livestock following delivery to a slaughter plant, including body condition score, lameness, foot, leg and body lesions, number dead on arrival, soiling, and carcass bruising (Grandin, 2010). Although most of these assessments are performed ante-mortem, bruising currently cannot be identified until after slaughter. Regulatory authorities such as the Canadian Food Inspection Agency, the United States Department of Agriculture, and Department of Environment, Food and Rural Affairs (UK) have expressed interest in the development of reliable quantitative tools to detect stress, pain and subclinical or non-visible injuries in food animals including horses following transport and prior to slaughter (Gloster *et al.*, 2011; Rainwater-Lovett *et al.*, 2009). The development of DT as a semi quantitative tool has the potential to fulfill this role. To achieve this goal, understanding the operator and animal related factors which affect skin temperature measured by DT under different environmental conditions is important in preparation for its use in the evaluation of animal welfare in field conditions. The objective of this study was to examine in clinically normal horses at rest, the effects of the following on skin temperatures as measured by DT

- Varied distances from which reliable thermographic images may be expected to be obtained under field conditions;
- Variations in skin temperatures among anatomic regions of interest (ROIs);
- Right versus left sided symmetry; and
- Environmental conditions (indoor versus outdoor).

4.2. MATERIALS AND METHODS

4.2.1. Animals

Eight clinically healthy Standard bred horses (five mares and three geldings) between 5 and 22 years of age (median 11 years) were used for this study following the approval of the Animal Care Committee at the University of Prince Edward Island (UPEI).

4.2.2. DT image acquisition

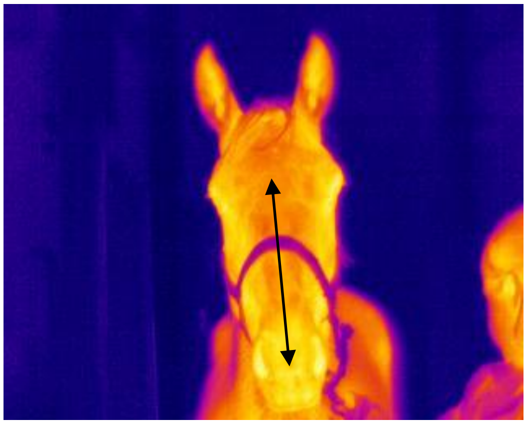
Digital infrared thermography data was obtained for each horse in standardized indoor and outdoor locations at UPEI with an infrared thermographic camera (ThermaCAM SC 2000 thermographic camera, FLIR system Inc., Wilsonville, OR 97070). All indoor images were taken during a single week when the animals were at rest and normally housed inside the Atlantic Veterinary College premises (temperature range 20 to 24 ºC; humidity range 20 to 58 %). All outdoor images were obtained when the same animals were at rest in an immediately adjacent small paddock measuring 7 m^2 (temperature range 19 to 22ºC; humidity range 40 to 53 %) on a different week (four weeks apart). The environmental temperature, relative humidity – measured using a hygrometer (Magna-Temp RH%, Canadian Tire, Toronto, ON) and distance between camera and the horse were measured using a laser distance meter (Master craft ® Hawk eye laser, Model no: LM04CN, Canadian Tire, Toronto, ON) during the capture of each image. The DT image with the correlated temperature data map generated by the camera hardware were saved and downloaded on to a computer equipped with software (ThermaCAM Researcher Professional 2.8 SR-3 software, FLIR system Inc., Wilsonville, OR 97070). Skin temperature readings were adjusted using this software for an object parameter emissivity value of 0.95 (MacPhee, 2008), distance between the horse and camera, environmental temperature and relative humidity.

To evaluate the effect of distance from the horse (an operator factor) on DT skin temperature measurements, thermographic images were obtained for each horse under indoor and outdoor conditions for the following ROI's: anterior view of the head region at 4 and 5 metres; left lateral view of the trunk region at 5 and 6 metres; right lateral view of the trunk region at 5 and 6 metres; left lateral view of the left front limb (carpus to hoof) at 1 and 2 metres, and; posterior view of the buttock region at a distance of 4 metres. Distances were chosen based on the field of vision attainable using the DT camera, and on expected field conditions anticipated at slaughter plants processing horses where close proximity to the horse is usually not be possible.

4.2.3. Data and statistical analysis

Mean skin temperature was calculated for each image from DT data points over a straight line drawn between two anatomic points, for each anatomic region. The thermography software was used to calculate the mean skin temperature by selecting sample temperature at several points (depending on the image pixel density) along the anatomical line chosen. The mean temperature of each head image was obtained over a line drawn from mid-eye level to the middle of the bridge of the nose between the external nares (Figure 4.1a and 4.1b). The mean temperature of each trunk image was obtained over a line drawn from the highest point of the withers to the lowest point of the flank (Figure 4.2a and 4.2b). The mean front limb temperature was measured over a line drawn from the mid-carpus level to the mid-fetlock joint (Figure 4.3a and 4.3b).The mean temperature of each image of the gluteal/ caudal thigh region was obtained over a line drawn between the level of tuber ischium (buttock) downwards to the lower thigh (where the semimembranosus and gracilus muscles intersect) (Figure 4.4).

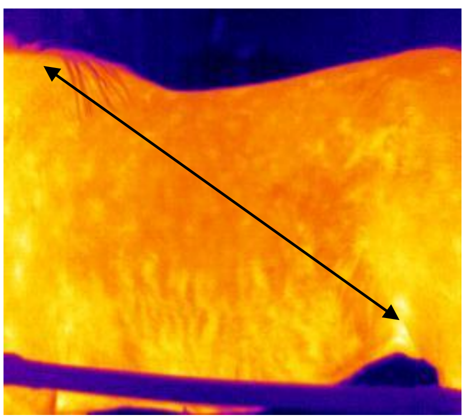
The mean skin temperatures measured under different conditions were calculated. The effects of the camera distance from the horse, different environmental conditions (indoor and outdoor), symmetry (left or right) and ROI were first evaluated unconditionally, and then modelled together in a multiple regression model to understand the effect of each variable on the mean skin temperature measured. To account for repeated measures, a mixed model regression analysis approach was employed, with the animal (subject) as a random effect (Dohoo *et al.*, 2009). Non significant effects ($P \geq 0.05$) were dropped in the final model and for others parameter estimates and predicted medians were obtained. In order to meet model assumptions (residuals to be normally distributed), the outcome (skin temperature) was log- transformed after subtraction of an offset value (23º C) determined by a Box-Cox type analysis (Venables and Ripley, 2001). Residuals of the model were checked for normal distribution and heteroskedasticity using graphical methods. Post estimation pairwise comparison of temperatures was performed between indoor and outdoor conditions for each ROI. The significance was set at $P<0.05$. Statistical analyses were carried out using the software (Stata 12.1, StataCorp, 4905 Lakeway Drive College Station, Texas 77845, USA.), except for the Box-Cox analysis which utilized the MASS library for R software (R foundation, Vienna, http://www.r-project.org/).

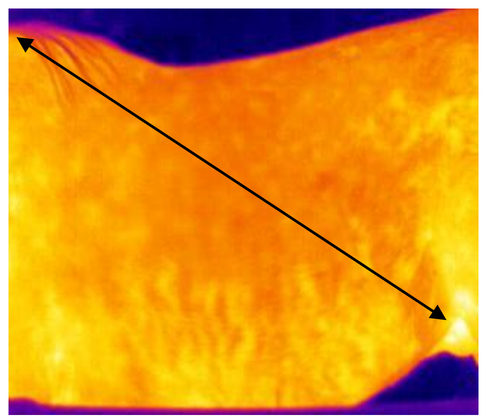


4.1a: Five metres distance image

4.1b: Four metres distance image

Figure 4.1a and 4.1b: Digital infrared thermographic images of the frontal head region at four and five metres, and the line between selected anatomical landmarks (mid-eye level to mid-point between the external nares) over which mean skin temperatures were calculated.

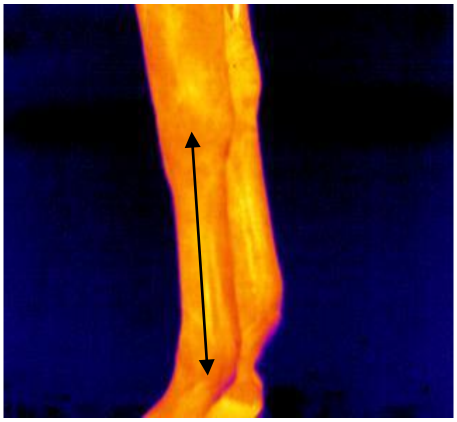




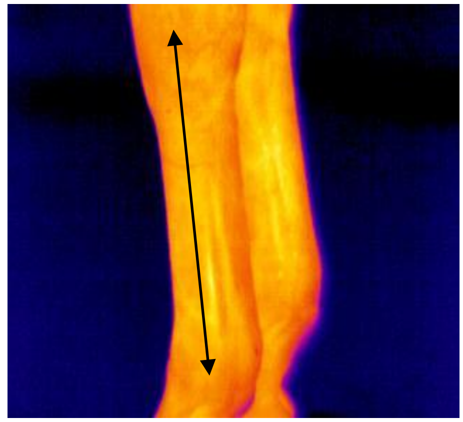
4.2a: Six metres distance

4.2b: Five metres distance

Figure 4.2a and 4.2b: Digital infrared thermographic images of the lateral trunk of a horse taken from a distance of 5 and 6 m. The line shows the span between selected anatomical landmarks (highest point of withers to the lowest point of the flank) over which mean temperatures were calculated.



4.3a: Two metres distance



4.3b: One metre distance

Figure 4.3a and 4.3b: Digital infrared thermographic images of the left lateral limb of a horse taken from a distance of 1 and 2 m. The line drawn between two anatomical landmarks (mid carpus and fetlock joint) shows the landmarks over which mean temperatures were calculated.

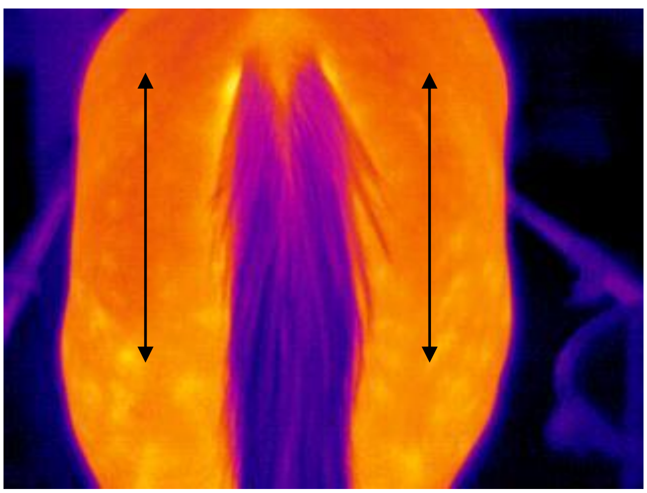


Figure 4.4: Digital infrared thermographic image of the posterior gluteal/caudal thigh region of a horse taken from a distance of 4 m. The line drawn between two anatomical landmarks (level of tuber ischium to level where the semimembranosus and gracilus muscles intersect (lower thigh) shows the landmarks over which mean temperatures were calculated.

4.3. RESULTS

Median and interquartile range of skin temperatures with respect to distance, ROI, symmetry and environmental conditions are listed in Table 4.1. Descriptive statistical mean skin temperatures calculated and statistical estimates from the model for ROI's under outdoor conditions were highest in the trunk region, followed by gluteal/caudal thigh regions, limb and head regions as illustrated in Table 4.1 and Figure 4.5 respectively.

The ROI's selected and environmental conditions significantly affected mean skin temperatures as measured by DT (Table 4.2). Skin temperatures under outdoor conditions were significantly different among the ROI's examined except between the head and limb regions. However, there were no significant differences in the skin temperatures among different ROI's under indoor conditions.

Distance at which DT imaging was performed did not have a significant effect on skin temperatures as measured by DT at the ranges compared. Symmetry (left or right side of body) did not have any significant effect on the skin temperatures calculated using DT. There were significant interactions between ROI's and whether thermography was performed indoors or outdoors. Pairwise comparisons of each ROI's (4 groups) between indoor and outdoor environments (2 groups) showed that all regions except the head had significantly higher temperatures when DT was performed under outside conditions (Figure 4.5).

Table 4.1: Median and interquartile range of skin temperatures measured at four regions of interest (ROI) on eight resting healthy horses by digital infrared thermography tabulated by distance, region of interest (ROI), symmetry and environmental conditions.

Factors	ROI	n*	Median skin temperature (interquartile range)ºC	
			Indoor	**Outdoor**
Distance (m)				
1	Limb	8	32.3 (30.1-32.9)	32.5 (31.2-34.8)
2	Limb	8	32.4 (29.6-32.9)	31.8 (29.9-34.6)
4	Head	8	31.8 (30.1-32.9)	31.5 (31.2-32.4)
5	Head	8	31.9 (30.6-32.8)	31.9 (31.0-32.3)
4	Gluteus	16	32.6 (30.4-33.3)	35.3 (34.3-36.8)
5	Trunk	16	31.7 (30.3-31.7)	36.3 (35.4-41.4)
6	Trunk	16	31.9 (30.2-32.6)	36.3 (34.7-40.9)
Symmetry				
Left	Trunk	16	31.7 (30.2-32.6)	35.8 (34.9-39.8)
Right	Trunk	16	31.7 (30.1-32.7)	37.4 (35.1-41.1)
Left	Gluteus	8	32.6 (30.3-33.0)	35.3 (34.3-36.4)
Right	Gluteus	8	32.7 (31.7-33.4)	35.6 (33.9-36.9))

n*= no. of DI images, m=metre/s, C= Celsius

Table 4.2: Parameter estimates with standard error of the coefficients (SE) and P value for the mixed model regression analysis between log transformed skin temperature measurements using DT and predictor: region of interest (ROI) and environment. Overall model significance by Wald test was P<0.001.

Predictor	Levels	Parameter estimates (log scale) (°C)	SE	P value
ROI -Indoor	Head	ref	n/a	n/a
	Trunk	-0.01	0.05	ns
	Gluteal/caudal thigh	0.07	0.06	ns
	Limb	0.01	0.06	ns
Environment	Indoor environment	ref	n/a	n/a
	Outdoor environment	0.04	0.06	ns
ROI ***x*** Environment	Head ***x*** outside	ref	n/a	n/a
	Trunk ***x*** outside	0.52	0.07	<0.001†
	Gluteal ***x*** outside	0.31	0.08	<0.001†
	Limb ***x*** outside	0.09	0.08	ns
Constant		2.1	0.06	<0.001

ref = reference level in the model; ns = not significant; ***x*** = interaction term; † significant interaction at the level of P<0.05; n/a = not applicable.

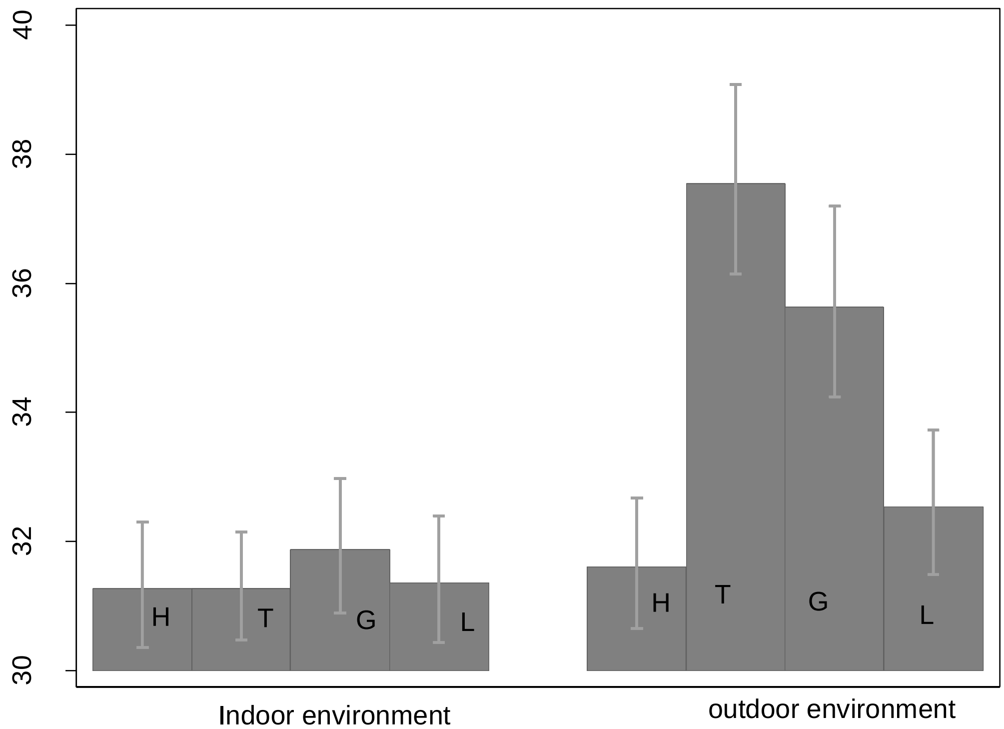


Figure 4.5: Model based predicted median and confidence interval of temperatures for each region (H=head, T=trunk, G=gluteal/caudal thigh, L=limb) under indoor and outdoor environments. The estimates were back transformed to the original scale (º C) from the modelled data summarized in Table 4.2.

4.4. DISCUSSION

Previous studies of DT in cattle and dogs have indicated that different ROIs of the same animal can have varying skin temperatures as measured by DT (Alsaaod and Büscher, 2012; Loughin and Marino, 2007). In the current study under indoor conditions, the posterior gluteal/caudal thigh region had an elevated mean skin temperature compared with other regions, even though this was not significantly different (Table 4.1). This elevated mean skin temperature was not unexpected. There are significant variations in skin thickness and subcutaneous vasculature among regions, resulting in areas where the outer skin surface is in close proximity to blood vessels, producing higher mean skin temperatures (Head and Dyson, 2001). Different parts of the body may also respond differently to the sympathetic nervous system, resulting in varying amounts of vasoconstriction and vasodilatation, subsequently affecting local blood flow, and thereby skin temperatures of ROIs (Loughin and Marino, 2007). However, in this study, the lack of significant difference in skin temperature among three regions (head, trunk and limbs) in the indoor environment indicated that the interaction with the environment may be a more critical determinant of skin temperature variability (Alsaaod and Büscher 2012).

Lack of significant regional skin temperature differences in normal horses using DT under indoor conditions has not, to the authors' knowledge, been reported previously. However, a similar study in healthy humans under indoor conditions resulted in similar findings among mean skin temperatures for the trunk and cheek regions (Zaproudina *et al.*, 2008). However, the extremities of human body (the feet) had different skin temperatures that were attributed to differences in circulation and tissue metabolism (Zaproudina *et al.*, 2008). Recording of stable mean skin temperatures in different ROIs of healthy horses using DT under indoor conditions in this study supports the use of this tool for evaluation of pathologies which may increase surface temperature, such as infection, inflammation, pain and stress (Weil et al., 1998; Turner, 2001).

The use of DT in an outdoor environment had an effect on skin temperatures measured by DT in this study. This supports the findings of others, where heat loss from different regions, for example the neck region versus the trunk region, has been shown to differ significantly in outdoor conditions (Autio *et al.*, 2006; Autio *et al.*, 2007). Higher temperatures in the trunk region are reflective of greater rates of heat loss under outdoor conditions (Autio *et al.,* 2006). Although the horses in the current study were stationary when evaluated by DT, it should also be noted that muscular activity may be greater when animals have the opportunity to move around, resulting in greater heat production by muscle as a by-product of increased metabolic activity (Ousey *et al*., 1992). This may be a more important factor in outdoor environments such as unloading or lairage areas, where the horse may have more opportunity to move around. Increased heat production in muscles increases the body's core body temperature. In such a situation, heat generated in the muscles may be transported to skin by blood (Hinchcliff *et al.*, 2008) thereby increasing DT temperature readings. For this reason the authors suggest that optimally, horses should be provided with a period of rest or equilibration prior to assessment in outdoor environments (Levet *et al.,* 2009).

Another plausible factor that has been suggested to account for regional differences in skin temperature is exposure to direct sunlight (Palmer, 1981) in outdoor environments. However, under the temperate environmental conditions of the study, there was no evidence of thermal asymmetry that may have otherwise supported a role for this factor in the study environment. This finding was in agreement with the work of others (Palmer, 1983) where the left and right side buttock skin temperature measurements were not significantly different. This is an important finding with respect to finding pathologies in different body parts of the animal, where one side may usefully provide the basis of comparison for the other side.

Ambient temperature has been known to affect skin temperature measured under outdoor conditions in cattle and horses (Alsaaod and Büscher 2012; Autio *et al.* 2006; Autio *et al*., 2007). However, in one study, no changes in skin temperature were recorded when the air temperature dropped by 3.1°C, but a significant response was found when the air temperature dropped by 6.5°C (Ivana Knizkova *et al.*, 2002). In the current study, the difference between the ambient temperature of the indoor and outdoor conditions was minimal (<3º C). The outdoor and indoor temperature range during this study was within the thermo-neutral zone of horses unaccustomed to winter weather (5-25 ºC) where a minimal change in metabolic rate would be anticipated due to ambient temperature (Morgan, 1998).

Mean skin temperatures measured using DT at different distances of different regions (1m, 2m, 4m, 5m and 6m) did not differ significantly. The distances evaluated were chosen for practical considerations with the view that these reflected the variability that might be experienced while attempting to utilize DT under field conditions encountered in slaughter plants. Such conditions may include an auction yard sale, slaughter plant or large animal diagnostic situation. The power of infrared radiation is independent of the distance ($\boldsymbol{P} = \varepsilon\sigma A\ (T^4 - T_c^4)$), where $\boldsymbol{P}$ = net radiated (infrared) power, ε = emissivity; σ = Stefan–Boltzmann constant 5.6703×10^{-8} W/m^{2}K^4 ; A = radiating area; T^4 = temperature of radiator; T_c^4 = temperature of surroundings), but attenuation may occur as a result of atmospheric absorption of radiation, particularly in situations of high humidity (Olowofela *et al.*, 2011). In the current study, the degree of humidity appeared to have minimal impact on the data, and the variation in distances was apparently insufficient for the thermographic camera to record a significant difference in temperature. Larger discrepancies may occur with larger differences in distances from the target of interest, due to the finite pixel density of the camera’s infrared sensor, and changes in the relative area being assessed with respect to the Stefan-Boltzman equation. In such circumstances, each pixel represents the average temperature of greater focal area of the target as distance between camera and horse increases. Use of the camera at close quarters may be associated with a reduction in the impact of outliers by measuring skin temperature of a larger zone rather than a spot temperature measurement. However, under field conditions, proximity to the horse may increase the risk of injury to the operator, and stress on the horses due to closer proximity to unfamiliar personnel. The thermal camera and proprietary software used has inbuilt provisions to adjust the skin temperature measured according to the distance from which the images are taken, and it appears that experimental conditions of the current study did not exceed this capacity.

In this study, mean skin temperature of a vector across the ROIs was utilized rather than spot skin temperature measures. Recording mean skin temperature of a larger area of a particular region may provide more useful information than spot temperature particularly when DT is used for detection of bruising ante-mortem in livestock before slaughter. For example, a single spot measure of temperature may miss a smaller lesion in a particular ROI, whereas mean skin temperature of the whole ROI may detect the lesion with more sensitivity. However, the drawback in using mean skin temperature is that any outlier value for temperature would be less apparent as they will be averaged out. This drawback may be mitigated by using maximum and minimum skin temperature values, standard deviation or confidence interval of skin temperature.

One practical challenge experienced during this study related to usage of DT was the inconvenience of manually recording environmental temperature, humidity and distance during the process of obtaining each DT capture. The authors suggest that the incorporation of an inbuilt distance measuring tool, environmental temperature and humidity measuring capabilities would help operators to detect pathology with significant ease. This would enhance the handling of DT and make it a simpler and more user friendly diagnostic tool under outdoor conditions.

In conclusion, within the conditions of the study, the reliability of DT based skin temperature measurements was better under controlled indoor conditions than under outdoor conditions. To use DT to detect injuries under outdoor conditions such as slaughter plants more controlled studies at varied ambient temperatures should be conducted. Significant differences in skin temperature among ROI's was noticed when DT was performed under outdoor conditions. However, stability of skin temperatures was observed between left and right side of regions (thermal symmetry) even under outdoor conditions. This stability is useful when attempting to detect asymmetric pathology. The differences in DT results between indoor and outdoor conditions of the study were not unexpected. This work sought to develop a means to quantitatively assess them under the conditions of the study, and to identify where these differences might most critically impact on objective thermographic detection of abnormalities in the future application of this modality.

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CHAPTER 5

USE OF DIGITAL INFRARED THERMAL IMAGING TO DETECT ANTE-MORTEM BRUISING IN HORSES AT A SLAUGHTER PLANT

5.1. INTRODUCTION

Digital infrared thermography (DT) is a semi-quantitative, non-invasive imaging technique that involves recording superficial thermal emission patterns generated by the radiation of heat from an animal (Turner, 2001). The principle underpinning DT is that any object with a temperature greater than absolute zero emits electromagnetic radiation in the non-visible range of the spectrum (Masur and Herbut, 2006). Pathology adjacent to the skin surface of animals such as bruising or contusions can be inferred from subjective interpretation of colour changes in the DT image qualitatively, or by quantitatively comparing the distribution of skin temperatures in an affected area with that of an unaffected area (Roehl *et al.*, 2009). Thus, DT had the potential to serve as a useful non-invasive and ante-mortem tool to assess injuries, such as bruising, contusions or hyperaemic areas caused by abrasion in transported horses. This study's hypothesis is that physiologic response of tissues to traumatic injuries can alter intra-dermal and subcutaneous blood flows, and therefore change skin temperatures in affected regions of the horse when compared to other unaffected regions.

Transport of horses for slaughter and related management practices, such as loading, unloading and handling may cause externally visible and non-visible injuries due to trauma including fractures, swelling, excoriations, and bruising (Grandin *et al.*, 1999; Marlin *et al.*, 2011). Bruising was a significant welfare issue affecting 51% of horses transported for slaughter reported by one group of researchers (Grandin *et al.*, 1999). The application of DT has been found to be useful in the post mortem detection of blunt force injury in humans (Bernstein *et al*., 2013). Blunt force injury may create bruising caused by damage to blood vessels and is associated with inflammation and pain (Bariciak *et al.*, 2003). In horses, DT has recently been used to identify subcutaneous blood flow associated with normal and abnormal wound healing (Celeste *et al*., 2012).

Currently, the predominant method for diagnosing bruising after transport for slaughter in food animals is by carcass inspection following slaughter (Anderson and Horder, 1979). Previous studies have attributed bruising in horses intended for slaughter to risk factors associated with transportation (Grandin *et al*., 1999, Stull, 1999, Marlin *et al*., 2011). However, the lack of a suitable method to identify bruised horses ante-mortem limits the ability to differentiate bruises obtained during transportation from those obtained during slaughter plant management procedures (Sandström, 2009) or at various stages on-route to slaughter (e.g. auction markets). It is important to determine which stage of the slaughter process is responsible for bruising, so that the risk may be reduced at the transport level, the slaughter plant level or the auction market level. Reliable and non-invasive methods of detection of bruising ante-mortem are needed to accurately assess injury patterns in horses intended for slaughter.

The use of DT to non-invasively detect minor changes in skin temperature for diagnostic and management purposes in animal health continues to be an active area of research (Bowers *et al.*, 2009). Previously, DT has been used for qualitative evaluation of injury in equine veterinary practice (Eddy *et al.*, 2001; Fonseca *et al.*, 2006; Levet *et al.*, 2009; Yanmaz *et al.*, 2007; Knízková *et al.*, 2007). When tissue is damaged, a region of localized hypo-or hyper-perfusion of blood occurs around the injury area which is apparently detectable by DT (Levet *et al.*, 2009; Yanmaz *et al.*, 2007). In horses, DT has been used successfully to detect ligament and tendon injuries (Denoix, 1994), bone stress injuries and joint disease in areas of limited soft tissue coverage, muscular injuries and neurological injuries (Braverman, 1989; Ross, 1989), but little has been reported on the sensitivity or specificity of the modality. The use of DT has also been described as a qualitative aid in the evaluation of equine lameness (Turner, 1991).

A qualitative methodology using DT images to detect bruising may enable animal inspectors working in slaughter plants to identify horses with bruising ante-mortem. Therefore, the main objective of this study was to estimate the sensitivity and specificity of DT images as a diagnostic test in detecting bruising ante-mortem when compared to the standard test of post-mortem visual examination of bruising in carcasses. Another objective was to determine the relationship between bruising and skin temperature as measured by DT. To achieve this objective, maximum skin temperature measurements of the pelvic region of horses with and without bruising in the pelvic region were compared.

5.2. MATERIALS AND METHODS

This study used a FLIR digital thermography camera (Model i7, FLIR Systems, Inc. 27700 SW Parkway Ave, Wilsonville OR 97070, USA) to detect and assess possible non-visible bruising in horses sent for slaughter. DT images were obtained from horses on arrival at the lairage of a federally approved slaughter plant in Québec, Canada. The lairage was a covered building without any mechanical thermal regulation and closely followed the outdoor temperature. DT images were obtained from horses immediately on arrival at the lairage (thermal assessment) so that carcasses of the same horses could also be assessed for bruising (standard test to identify true positive). Horses that underwent longer durations of lairage were excluded from the study because of difficulty in identifying the carcass of the horse from which images were obtained. Ethical approval for this research was obtained from the Animal Care Committee of UPEI.

5.2.1. Methodology for bruising detection using DT images

DT images were obtained from 93 horses immediately before being moved into the stunning pen for slaughter. One left lateral image, one right lateral image and one caudal view of the pelvic area were obtained from each horse from an elevated platform 5 metres away from the animal. After stunning and skinning of the horses from which DT imaging was obtained, their carcasses were examined for bruising. Identified bruising was recorded using anatomical landmarks on two horse outline diagrams, one representing the left side and another right side of the horse. A bruise on the carcass was defined as the discoloured area on the carcass surface caused by damage to the blood vessels.

The thermographic camera used to obtain DT images was calibrated so that those areas on the skin surface of the horse with a temperature higher than a threshold temperature appeared as red patches on the DT image. The threshold temperature was calculated by using the FLIR camera to obtain spot skin temperature measurements for each sampled horse in the neck, dorsal pelvic, and flank regions from a distance of 5 metres (Figure 5.1). The three spot temperatures were averaged and that value was used as the threshold skin temperature for that particular horse to detect thermal anomalies (e.g. 23º C was used as the threshold temperature for the horse in Figure 5.3). The emissivity was set at 0.96 (MacPhee, 2008) for all DT images.

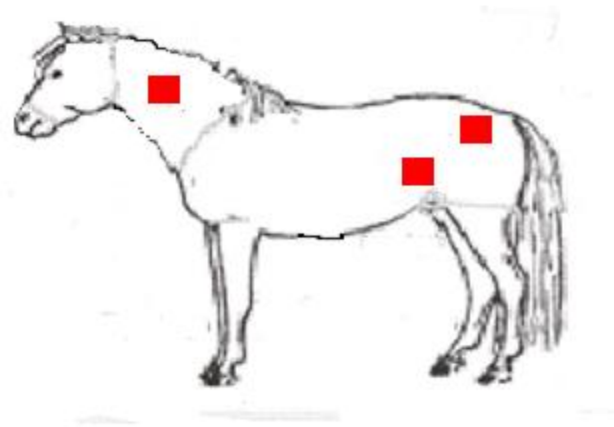

Figure 5.1: Diagram of left side of horse identifying the dorsal pelvic, neck and flank regions where spot skin temperatures were measured to obtain the threshold temperature for each horse based on the average of these three measurements.

DT images obtained were downloaded to a computer for detailed assessment using ThermaCAM Researcher software (FLIR Systems, Inc. 27700 SW Parkway Ave, Wilsonville OR 97070, USA). Not all regions of elevated thermal emission (red patches) were considered as potential bruising because some may be due to areas where the blood supply is normally greater than in the surrounding areas such as around the eyes. In order to eliminate these regions of physiologically increased emission from those associated with pathological skin temperature increase (possible bruising); the left lateral image (left side of the animal) was compared with right lateral image (right side of the animal). Thermal regions of interest that were asymmetrically present in only one side of the image or body were considered as potentially bruised. The pelvic image was used to refine the presence of bruising in the lateral regions. Regions of elevated thermal emission (red patches) that could potentially represent bruising (diagnostic test) were marked on two horse outline diagrams (to represent the left and right side of the body) so that comparisons could be made with the two similar horse outline diagrams of bruising identified by carcass assessment (standard test) according to anatomical landmarks. Therefore, each horse outline diagram indicating one side (left or right) of a horse was the unit of measurement (n=186). Comparison between the horse outline diagram representing abnormal patches in DT images and carcass bruising was done visually by anatomical landmarks. If an asymmetric thermal region of interest anatomically coincided with a carcass bruise marking, then that horse outline diagram (which represented one side of the horse) was considered positive for bruising (true positive). When one positive bruise was identified by DT, then other symmetric or asymmetric zones of increased thermal emission patches in the same horse outline diagram were disregarded thereby the unit of measurement could be reduced to two units per horse (left and right side). If there was one or more asymmetrical region of thermal emission in the horse outline diagram without a corresponding bruise/s in the carcass assessment horse outline diagram, then that diagram was considered a false positive. A horse outline diagram with no bruise in carcass and no asymmetrical region of thermal emission on DT was considered a true negative. A horse outline diagram with bruise in carcass which was not represented by an asymmetrical region of thermal emission on DT was considered a false negative.

5.2.2. Methodology to understand the relationship between bruising and skin temperature as measured by infrared thermography

The same 93 horses that were used for the qualitative assessment of bruising were subjected to a quantitative assessment of DT images by measuring the maximum recorded skin temperature of the pelvic area. As the prevalence of bruising was observed to be high in the pelvic and tail-head region (Chapter 6), DT images of the dorsal pelvic region were obtained from a 2 metre distance (Figure 5.2). DT images of pelvic region of only 68 horses of the 93 horses were sampled, were usable for quantitative assessment because of picture quality issues. The pelvic images were downloaded to the ThermaCAM researcher software (ThermaCAM Researcher Professional 2.8 SR-3 software, FLIR system Inc., Wilsonville, OR 97070). For each of the images, a vector was drawn connecting the two most prominent points of the tuber coxae (point of the hip) and tail head to form a triangle (ARO1 in Figure 5.2). The highest (maximum) skin temperature identified by DT in the selected area was recorded. As DT was performed in outdoor conditions, the ambient temperature was also recorded for each corresponding DT image. For each image, the presence or absence of carcass bruising detected post-mortem in pelvic region of that same horse was also recorded.

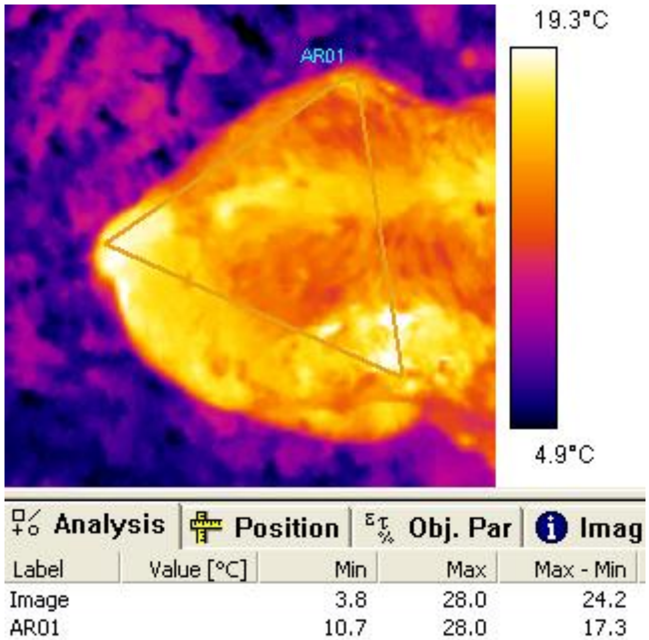

Figure 5.2: Thermal image of the pelvic region (ARO1) used to evaluate the relationship between maximum skin temperature recorded and presence of bruising or its absence. A maximum skin temperature of 28ºC was obtained from the ThermaCAM researcher software for this horse.

5.2.3. Statistical analysis

Data was entered in Epidata (EpiData Association 2000-2012;http://www.epidata.dk/index.htm) and transferred to Stata 12.1 statistical software for analyses (StataCorp, 4905 Lakeway, College Station, Texas 77845 USA). Evaluation of the threshold temperature was carried out by calculating the mean skin temperature for each animal. The percentage coefficient of variation of the mean was then plotted against the mean skin temperature. Sensitivity, specificity, and positive and negative predictive values were calculated for the qualitative assessment test for bruising at each horse outline diagram level. The carcass assessment for bruising was used as the standard test against which DT image assessment was compared. Positive and negative predicted values for various prevalence ranges were calculated and plotted. .

For quantitative assessments, a linear regression model was built with the maximum skin temperature of the pelvic region of each horse as an outcome variable. The predictors for the model were ambient temperature at the time of obtaining the images and whether the horse was bruised in the pelvic region. Linearity of the continuous variable (ambient temperature) was examined. Unconditional associations were checked for each variable before building a model. Residuals of the model were checked for normal distribution and heteroskedasticity using graphical methods.

5.3. RESULTS

5.3.1. Qualitative assessment

Figure 5.3 (‘a’ and ‘ b’) illustrates a DT image of a horse with possible bruising in the right flank region because there were two zones of increased thermal emission in the right side trunk image as compared to only one in the left side trunk image, indicating an asymmetry and thus a potential bruise. Figure 5.3 (c) illustrates the pelvic region of this animal had neither any zones of increased thermal emission (no area above the threshold temperature of 23ºC) nor any thermal asymmetry while comparing the left side of the pelvic area with that of the right side indicating bruising was not detected.

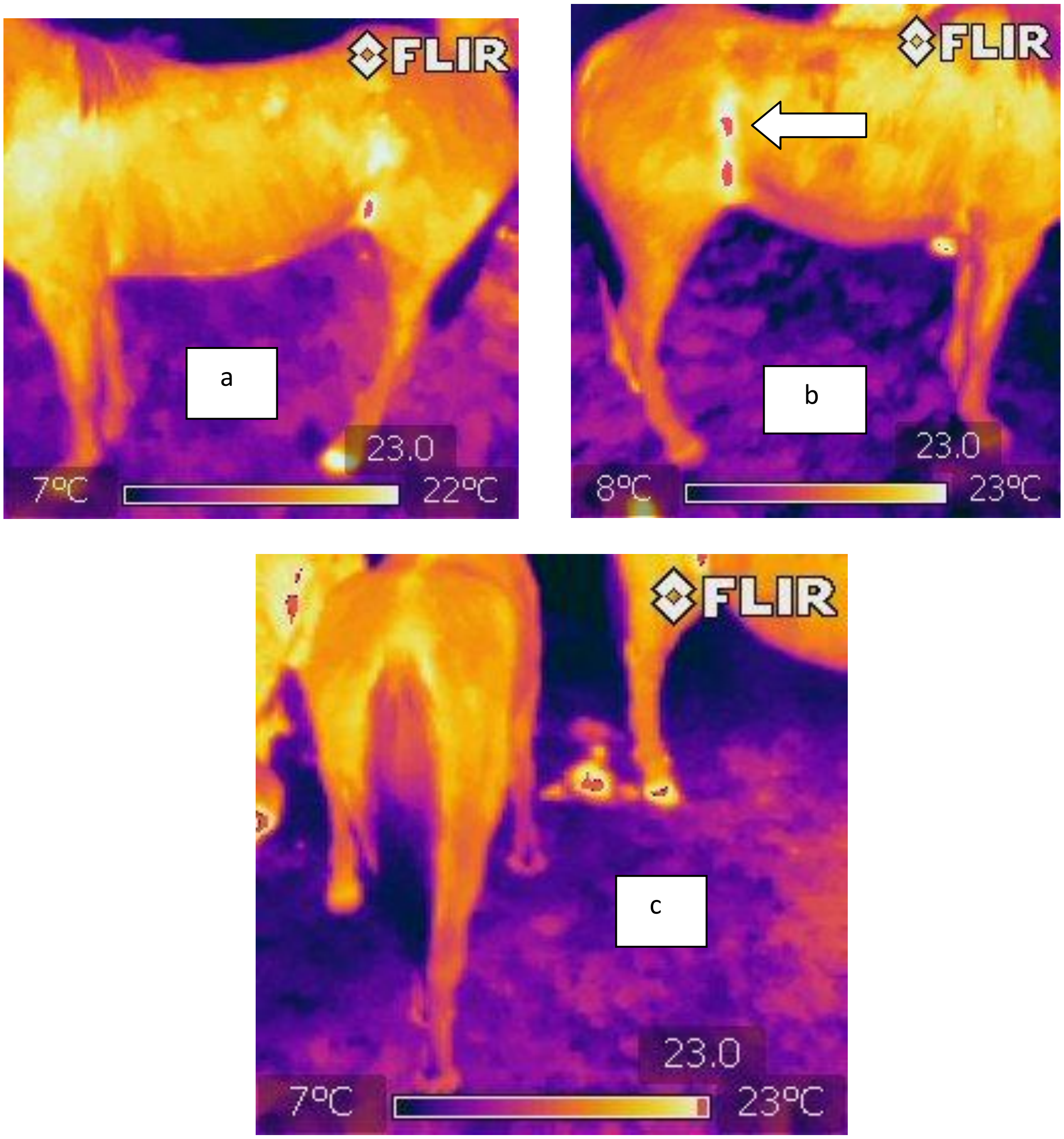

Figure 5.3: Left lateral (a) right lateral (b) and pelvic (c) DT images of the same horse acquired at a distance of 5 metres to detect potential bruising. Arrow in (b) shows an asymmetric high temperature zone (red “patch”) when compared to left lateral view (a) in the flank region. In this particular animal, the pelvic image (c) is symmetrical between left and right sides of the pelvis.

Comparisons between the visual carcass bruising lesions and DT methods of bruise identification at the region of interest level (left and right sides of body outline diagrams) of 93 horses (n=186) produced a sensitivity of 42% and specificity of 79% (Table 5.1). Positive predictive values (PPV) and negative predictive values (NPV) were calculated at different ranges of true prevalence and are plotted in Figure 5.4.

Table 5.1: Sensitivity, specificity, positive predictive value, and negative predictive value of digital infrared thermography as a tool to identify bruising (n=186).

Assessment of bruising by visual assessment of carcasses	Assessment by DT		
	Present	Absent	
Present	42 (true positive)	58 (false negative)	100
Absent	18 (false positive)	68 (true negative)	86
	60	126	186

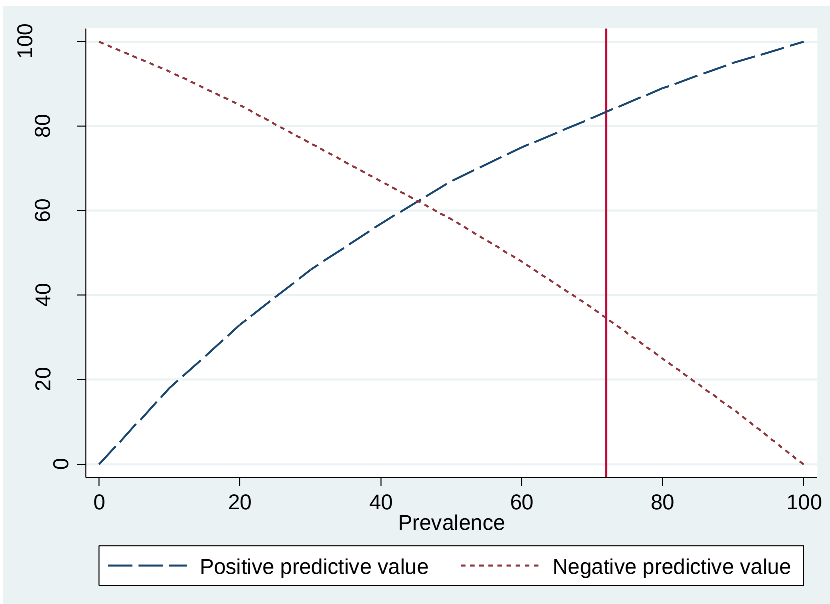

Figure 5.4: A plot of positive and negative predictive values versus prevalence for the DT based diagnostic test for bruising. The vertical line is the true prevalence (72%) at which the positive predictive value is 84% and the negative predictive value is 35%.

Evaluation of threshold temperature

Since the threshold temperature chosen in this study was the average of three spot skin temperature of different regions, estimation of the variation in skin temperature in different regions was essential to evaluate the precision of the threshold temperature. Evaluation of the threshold temperature was carried out by calculating the coefficient of variation and plotting it against the mean skin temperature for each animal. Since coefficient of variation can only be computed for ratio scales, skin temperatures measured on the Celsius scale were converted to Kelvin units. As coefficient of variation is a normalized measure of dispersion of a probability distribution, Figure 5.5 shows that the distribution was close to the mean skin temperature.

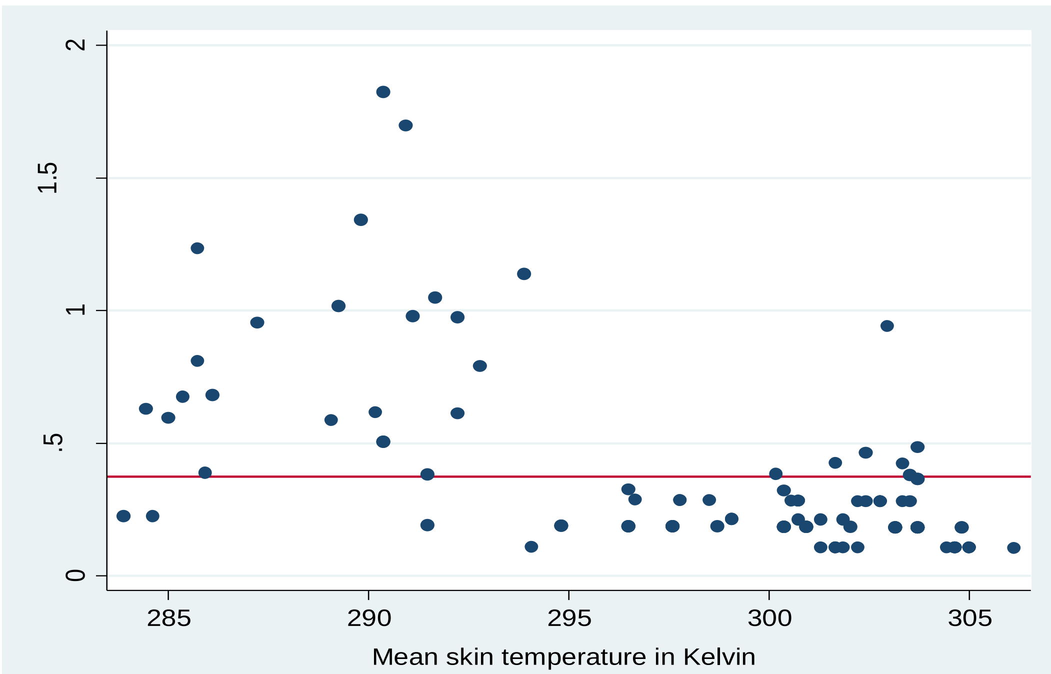

Figure 5.5: Coefficient of variation % plotted against threshold temperature in Kevin (threshold temperature was the mean of three spot skin temperature at neck, pelvis and flank). The horizontal red line indicates the mean skin temperature coefficient of variation.

5.3.2. Relationship between bruising and skin temperature as measured by infrared thermography

The maximum skin temperature of the pelvic region of horses without bruising by visual assessment of carcass was 34 .5±1.7ºC (mean and standard deviation, n=16) whereas the maximum skin temperature of the pelvic region selected in horses with bruising by visual assessment of carcass was 34 ±1.5 ºC (n=52). When association between ambient temperature and maximum skin temperature of the pelvic region was examined by an unconditional linear regression, every 1ºC elevation in ambient temperature increased the maximum skin temperature by 0.38ºC. Ambient temperature was significantly associated with maximum skin temperature in unconditional linear regression and also in multivariable linear models (Table 5.2). However, the maximum skin temperature of the pelvic region of bruised horses (n=52) was not significantly higher than that of non-bruised horses (n=16).

Table 5.2: Effect of ambient temperature and presence of bruising or its absence on maximum skin temperature of the pelvic region measured by digital infrared thermography evaluated by a linear multivariable model (R^2=0.24, P<0.005).

Predictor	Coefficient	P value	Confidence interval
Ambient temperature	0.30	<0.001	0.15 to 0.45
Not bruised	Reference range	Not applicable	Not applicable
Bruised	-0.39	0.43	- 0.9 to 1.4
Constant	28.5	<0.001	25.6 to 31.5

5.4. DISCUSSION

5.4.1. Qualitative assessment

In this study, using a qualitative ante-mortem approach, DT images were used to assess bruising by identifying body parts with elevated skin temperature. Thermal symmetry was used as an additional control in the methodology to improve the specificity to detect bruising, although it could reduce sensitivity. Asymmetry in skin temperature has been used as a diagnostic tool to detect foot lameness in dairy cattle (Alsaaod and Büscher, 2012; Whay *et al.*, 2004) and breast cancer in human beings (Kontos *et al.*, 2011). Alsaaod and Buscher (2012) and Whay *et al*. (2004) established that the skin temperature was higher in the affected limb (lame limb with foot problems) than in the contra lateral normal limb in dairy cattle. Both studies suggested that the same animal may act as its own control to detect pathology. However, using thermal symmetry as an additional protocol in the methodology (not used in Chapter 6) resulted in a relatively modest specificity of 79% to detect bruising.

Plausible reasons for false negatives in this study are, the ambient temperature during transport, which was very low on some days during winter (-20ºC) and not providing equilibrium time for surface temperature to stabilize after exposure to cold. Plausible reasons for false positives are skin wounds (e.g. abrasions) without bruising, physical contact with other horses increasing surface temperature and hyperaemic areas which were not traumatized enough to cause carcass bruising (Autio *et al*., 2006). Thirty three % of this sample population had visually observable skin injuries (Chapter 6). Contact with the vehicle can have had an effect by increasing or decreasing the skin temperature of the animal in specific locations because metal body can be cold during winter.

Choosing a threshold temperature was an important step when using DT qualitatively under outdoor conditions. Under indoor conditions (when housed in thermo-neutral zone) normal skin temperature of a horse will be about 5ºC cooler than the core temperature (Turner, 2001) i.e. around 32ºC. Hence, under indoor thermo-neutral conditions, 32ºC may be chosen as the threshold temperature to detect possible hyperaemic areas. However, since this study was undertaken in outdoor conditions, the skin temperature of horses are likely to have differed according to the ambient temperature (Alsaaod and Büscher, 2012; Knizkova *et al.*, 2002). Hence, a flexible threshold temperature should be established for each examination to detect thermal anomalies that may be indicative of bruising or other pathology. The coefficient of variation graph (Figure 5.4) indicated that the variability of threshold temperature used in this study for each animal was not large. The use of three spot skin temperature points to find the threshold temperature had sufficient precision (with the exception of a few outliers) and was validated as a suitable approach to establish threshold under field conditions. Therefore, this methodology to identify threshold temperature for each animal has practical application when DT technology is used for diagnostic purposes under outdoor conditions.

The qualitative diagnostic approach using DT images to detect bruising had a positive predictive value of 70% (the probability of the horse being identified using DT as bruised when they are truly bruised). Predictive values are highly influenced by the level of prevalence (Figure 5.5). At the true prevalence of 72% bruising (true prevalence as estimated in chapter 6) the positive predictive value was 84%, however, the negative predictive value had fallen to 35% at this prevalence. True prevalence of 72% from Chapter 6 is used because it was considered more representative of the population. When the prevalence of bruising is high, a positive DT image is most likely a true positive, but a negative image is, in most cases underestimates the level of bruising in a group of horses.

Ideally, animal inspectors or welfare assessors evaluating the welfare status of horses at the pre-slaughter stage would like to have a simple diagnostic tool to identify horses with bruising using a practical screening test. To achieve this objective, the DT images should have high sensitivity thereby detecting all bruised horses. However, in this study the qualitative diagnostic test had low sensitivity. This finding was consistent with the finding of Kontos *et al*. (2010), when they used DT by qualitative method to detect breast cancer in humans.

A high proportion of bruising as detected by visual assessment of carcasses was not detected by the DT which was indicated by the high number of false negatives (31%). Biologically, horses that have bruising should show some inflammation around the bruising and hence should be detected by DT (Bariciak *et al.*, 2003). There are two ways by which false negatives could be reduced. One way is by reducing the preset threshold temperature value thereby increasing the sensitivity. The second way could be to provide an equilibration time for horses after transportation and before DT images are obtained, in order for them to recover from the effects of transport (Tunley and Henson 2004; Gloster *et al*., 2011). It is possible that transport could cause an increase in skin temperature of horses in some particular regions. Horses that went for slaughter immediately after unloading were purposefully chosen for the study to facilitate easy follow up of the same animal after stunning for carcass examination. If there had been some equilibration time for these horses to settle down in the lairage, then the skin temperature of the horses might have reached a plateau (Tunley and Henson, 2004) thereby increasing the sensitivity to detect pathology, such as bruising.

5.4.2. Relationship between bruising and skin temperature as measured by infrared thermography

The maximum skin temperature of the pelvic region of horses that were bruised was not significantly different from that of horses that were not bruised. This finding suggests that horses with bruising may not be the only ones with increased skin temperatures in the pelvic region. There are two factors may have contributed to this outcome. Firstly, the sample size of non-bruised horses was low (n=16) which may have been insufficient for statistically significant comparison. Second, the infrared thermography camera (FLIR Camera, Model i7) used in this study, although economically priced had modest accuracy with an error in accuracy of ±2ºC. Other overriding factors such as physical activity (Wallsten *et al*., 2012) and close contact with other horses may act as confounding factors increasing the skin temperature of horses, meaning that bruised horses are not the only ones with increased thermal emission from the surface. In the current study, only the pelvic region was evaluated because different regions of the horse may have significantly different skin temperatures; hence only comparisons of the same region can be performed by this method. As only the skin temperature in the pelvic region was studied, there is the possibility that bruising in other areas had characteristics that might be more directly related to localised skin temperature increases than those studied in the pelvic area.

Based on the results of the current study, the relationship between ambient temperature and skin temperature as measured by DT was linear. For every 1º C increase in ambient temperature, there was a 0.38ºC increase in skin temperature. A similar study in dairy cows predicted a similar linear relationship (Alsaaod and Büscher, 2012). Understanding of this relationship may be of value in future studies where DT is used in outdoor conditions, in particular can be used as a correction factor to adjust for ambient temperature.

5.4.3. Limitations and conclusions

Many difficulties were encountered during this study with respect to obtaining the DT images for the sampled horses. Because the horses were not handled or restrained, good quality images of all three DT images for qualitative assessment and the pelvic view DT image for the quantitative assessment was difficult to obtain in a standardised position. Horses in the lairage of this slaughter plant are not restrained by handlers, and free to move around. Incorrect positioning may have an effect on the angle of the images obtained, which in turn could have had an effect on the skin temperatures measured. The working pace in the slaughter plant was fast, and there was little time available to get good quality images, particularly if the image quality was poor after the first attempt and a second image was needed. Similar challenges were encountered by Sandström (2009) while developing a monitoring system for the assessment of cattle at slaughter plants.

In conclusion, the DT image has a sensitivity of 42% for identifying bruising and a specificity of 79% for correctly identifying images with no bruising. One way to improve the screening ability of DT image technology to detect bruising may to use a more sensitive camera that has better accuracy regarding skin temperature measurement.

Increasing the equilibration time provided for the horses after transport and controlling the ambient temperature in the lairage to be maintained in the thermo-neutral range for horses may also increase the sensitivity of this approach. More studies are needed to better understand the relationship between ambient temperature and horse skin temperature as measured by DT, so that a better threshold temperature can be set to increase sensitivity and specificity in the detection of bruising.

Research to improve this methodology may help regulatory authorities' e.g. Canadian Food Inspection Agency and United States Department of Agriculture to detect invisible injuries in the live animals at different stages of food animal production. In particular, this could be helpful in the enforcement of regulations regarding transportation of horses, horse handling guidelines and management practices at the slaughter plant. Additionally, it may assist in identifying risk factors for injuries during food animal transportation. It also could help the meat industry to self-regulate in terms of animal welfare issues, and to reduce their economic loss by developing procedures and guidelines to prevent injury.

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CHAPTER 6

WELFARE ISSUES ASSOCIATED WITH THE TRANSPORT OF HORSES TO SLAUGHTER IN CANADA

6.1. INTRODUCTION

In Canada and the United States of America (USA), annually an estimated 100,000 horses that have reached the end of their productive use for recreation or sport are sent for slaughter for human consumption (McGee *et al.*, 2001; Santschi, 2011; Agriculture and Agri-Food Canada 2011). Slaughter of horses for meat provides an effective option for reducing the number of unwanted horses, which might otherwise suffer due to neglect (Leadon, 2012; Messer, 2004; Stull, 2012). However, management procedures for slaughter may also cause concerns in terms of welfare, due to the long journeys that some of these horses have to undertake before slaughter (Grandin *et al.*, 1999; Stull and Rodiek, 2000). It has been reported that since the stoppage of horse slaughter in the USA in 2007, horses are being transported longer distances to Canada and Mexico (Messer, 2004; Stull, 2008). There is little quantitative information published on the patterns of journey to slaughter plants in Canada, and the state of the horses on arrival at the slaughter plant. This study was designed to provide quantitative information on welfare issues associated with the transport of horses to slaughter, and also to better understand the association between the journey and welfare outcomes observed at the slaughter plant.

Welfare issues, such as injury (Grandin *et al.,* 1999; Marlin *et al.,* 2011), dehydration (Friend, 2000), hunger and fatigue (Gibbs and Friend, 2000; Stull, 1999) can occur as a consequence of long road journeys to slaughter. Estimation of the prevalence of these welfare issues and understanding the causal factors are important first steps to improving the welfare of horses transported for slaughter. Some welfare outcomes are easier to measure than others. For example, an unambiguous categorization of injuries can be made and thereby prevalence of injuries estimated in slaughter horses. However, for welfare issues such as thirst, fatigue and hunger, which are subjective sensations, estimation of prevalence is difficult even in humans who can self report (Bennett and Blissett, 2014). These welfare issues in animals which are subjective can only be assessed indirectly by measuring physiological, clinical and specific behavioural states and events thought to be associated with these states.

In 1998 and 1999, studies were conducted in the USA to estimate the prevalence of injuries in slaughter horses (Grandin *et al.*, 1999; Stull, 1999). The prevalence of injuries was estimated to be 2 to 8% depending on the classification of the injuries. These studies also indicated that the use of double deck trailers was one of the causative agents for the high prevalence of injuries. Based upon this finding, restrictive regulations on usage of double deck trailers for commercial horse transport in the USA were enacted (Shames, 2011). Apart from vehicle type, there have been other causal factors identified as being associated with injuries during the transport of slaughter horses. Horses may lose their balance and occasionally fall during transport (Collins *et al.*, 2000); social interactions, such as biting and kicking during transport, can also cause injury (Collins *et al.*, 2000; Grandin *et al.*, 1999). Fighting was identified by Grandin *et al*. (1999) as a major cause of injuries in horses transported for slaughter in the USA. Twelve and a half percent of carcasses bruises were attributed to bites and kicks that occurred due to fighting during transport and also during lairage (Grandin *et al.*, 1999). The type of vehicle used, maintenance of the vehicle (particularly the interior parts which come in contact with animals) and stocking density can have an effect on the incidence of injuries.

Dehydration and fatigue are welfare issues that have been reported in horses transported for long duration (Friend, 2000). Horses that were transported for long duration (more than 24 hours) in hot weather (higher than 29º C) without access to water showed signs of dehydration (raised serum osmolality and serum/plasma total protein concentration), behavioural signs of fatigue, and increased heart and respiration rate (Friend, 2000; Stull, 1999). In addition to journey duration and season (Stull and Rodiek, 2000), other factors that have been reported to have affected the levels of dehydration in horses during transport are: rest period provided during transport with or without access to feed and water (Stull *et al*., 2008), pre-existing health conditions (Grandin *et al.*, 1999) and watering and feeding facilities during transport (Friend, 2000).

Pre-existing health conditions in horses transported for slaughter can have a significant impact on welfare (Grandin *et al.*, 1999; McGee *et al.*, 2001). A survey by Grandin *et al*. (1998) found that 1.5% of horses transported for slaughter were considered unfit for transport because of fractures, emaciation, or lameness, or were dead on or shortly after arrival. According to this study, 8% of the horses were classified as experiencing severe welfare problems, with the majority of them being emaciated and chronically lame, which indicated long term neglect by animal owners rather than welfare issues arising due to transport.

The present epidemiological study on welfare issues related to the transport of slaughter horses aimed to provide reliable, field-level data to understand the prevalence of welfare issues and also help to understand the risk factors associated with them. Second, the results from this research study can be used when formulating guidelines for best practices to avoid unnecessary suffering (Reece *et al.*, 2000). Third, they can serve to achieve evidence-based resource allocation within the horse slaughter industry, better quality assurance, and improved efficiency and productivity. Fourth, they can be used to mitigate risk factors and address public concern of welfare issues.

6.1.1. Objectives

The objectives of this study were as follows:

1. Quantify the types of vehicle used, source and journey duration of horses arriving at a Canadian slaughter plant.

2. Assess the welfare of horses on arrival at the slaughter plant by quantifying injuries, physiological and clinical measures.

3. Assess the prevalence of pre-existing conditions that might have been associated with pain and discomfort.

3. Observe the horses at lairage for behaviours that indicate hunger and thirst; and also behaviours that predispose horses to injury.

4. Examine relationships between journey characteristics and welfare assessments.

6.2. MATERIAL AND METHODS

6.2.1. Transport and slaughter management

This study was conducted in Québec, in one of the four federally approved Canadian slaughter plants. All the trailers used for the transport of horses were single deck livestock trailers, except for a few trailers that originated from within Canada (pot bellied), and these were excluded from this study sample. The size of the trailer containers (all those from the USA) had similar dimensions; length and breadth were 16 m x 2.3 m. These trailers were divided into three or four compartments. Stallions were always loaded in one or two of the small sized compartments located in the front portion of the trailer with individual partitions. The remaining bigger compartments had mares and geldings in groups. All truckloads from the USA came from the following five states; Pennsylvania, Ohio, Iowa, Indiana and Kentucky. All truckloads from Canada came from the province of Ontario.

Enquiry from the truck drivers indicated that horses were not fed or watered during transport; however, some truckloads had on-board rest periods for the horses as the truck needed to be stopped for inspections by border security agents and also for driver rest periods.

Once the truckload reached the slaughter plant, the horses were unloaded into a lairage area that had 14 pens (four large sized pens (8.48 x 4.24 m), eight small sized pens (4.24 x 2.20 m), and two single enclosures for stallions). Ante-mortem inspections were conducted in these lairage pens by the slaughter plant officials and Canadian Food Inspection Agency officials. The lairage pens had bedding (wood shavings) for horses;

automatically filling watering facilities and restricted access to hay (limited amount of hay given infrequently) during lairage. Some horses were slaughtered immediately upon arrival whereas others were lairaged up to two days, particularly on weekends. After ante-mortem inspection, the horses were driven using a whip (mostly by creating noise and sometimes with direct contact) through a rail system into the stunning pen. The lairage pens had been built using a design recommended by Grandin (1991) so that minimum intervention was needed by handlers to drive the horses from lairage to the stunning pen. When horses reached the stunning pen, they were stunned by captive bolt and then hoisted for exsanguination and skinning.

Data collection for this study was undertaken in two phases. First, starting from the summer months of 2011 (June to September) and again during the following winter months of 2012 (January and February). Between the summer season and the subsequent winter period, renovation work was carried out in the lairage and stunning pen (anti-slip ground surface upgrade).

6.2.2. Study methodology

6.2.2.1. Journey characteristics and initial health assessment of all horses

One hundred and fifty truckloads of horses were studied to quantify the welfare issues and understand associated risk factors for transport of horses for slaughter (n=3940 horses). Determination of this sample size was performed using the preliminary data from 15 truckloads (subset of the 150) (Figure 6.1). The mean log proportion of horses injured per load at short journey durations (<20 hours) and long journey durations (>20 hours) were -2.35 and -2.58 with standard deviation of 0.506 and 0.543 respectively from the pilot study. Hence, at the power of 0.8 and alpha 0.05, the sample size required was 164. These 150 truckloads represented approximately 90% of truckloads those which arrived on the data collection days and approximately six percent of the truckloads which normally arrived at this slaughter plant in a year. Ninety one truckloads were observed in the summer sampling period and 59 truckloads were observed in winter months. Ethical approval for this study was obtained from the University of Prince Edward Island Animal Care Committee.

Details regarding journey characteristics (place of origin and journey duration) were obtained for all the 150 truckloads from the shipper certificates and interviews with the drivers or slaughter plant employees. Journey duration was determined from the declaration on the shipper certificates for the time of loading at the auction market or other horse collecting centre until the time of unloading at the slaughter plant. For 79 of the 150 truckloads, additional load characteristics were obtained from the slaughter plant (total weight of horses in the load, average horse weight in the load and average carcass yield). Assumed stocking density in the vehicle was calculated by dividing the total weight of horses in each load by the total area of the vehicle available for the horses. The stocking density calculation was made under the assumption that the distribution of horses in each compartment of the trailer was similar.

When horses were unloaded from the trailers at the slaughter plant, all horses from each load (n=3940) were visually examined for health issues and the following welfare issues were recorded:

a. Number of horses with skin injury(s).

(An injury was defined as skin that had been abraded (scraped to produce a hairless patch), torn, pierced, cut, or otherwise broken).

b. Number of horses with injury(s) on the head region (subset of 'a') (Figure 6.2).
c. Number of horses with swelling(s) around the eye region.
d. Number of horses with body condition less than 3 (on a scale of 1 to 5) (Burn *et al.*, 2010a,b)
e. Number of horses with pre-existing health conditions

(Pre-existing health condition was defined as horses with visible deformity (asymmetry) in body parts, the presence of large granulating wounds or any abnormal growth visible on their body).

f. Number of horses that arrived in a non-ambulatory condition

(Non- ambulatory was defined as a horse lying down inside the vehicle on arrival that could not stand up and walk into the lairage).

All of the above assessments ("a" to "e") were performed after the horses had been unloaded and were walking from the vehicle to the lairage (except non-ambulatory condition). The horses were observed from an elevated platform so that the whole body of the horse was visible.

Body condition score was scored at a distance and without palpation using the following 5-point scale adapted from the work of Burn *et al.* (2010a, b) and Pritchard *et al.* (2005);

- 1 = Ribs, spines and hip bones prominent, top line of neck concave and pelvis hollow, 2 =Spine and hipbones visible, neck flat, pelvis/ribs flat,
- 3 =Pelvis and spine just visible, neck top line straight,
- 4 = Rounded pelvis, spine not visible, neck top line slightly convex, and
- 5 = Ribs, spine and pelvis not visible, top line of neck convex, pelvis rounded with 'gutter' along spine.

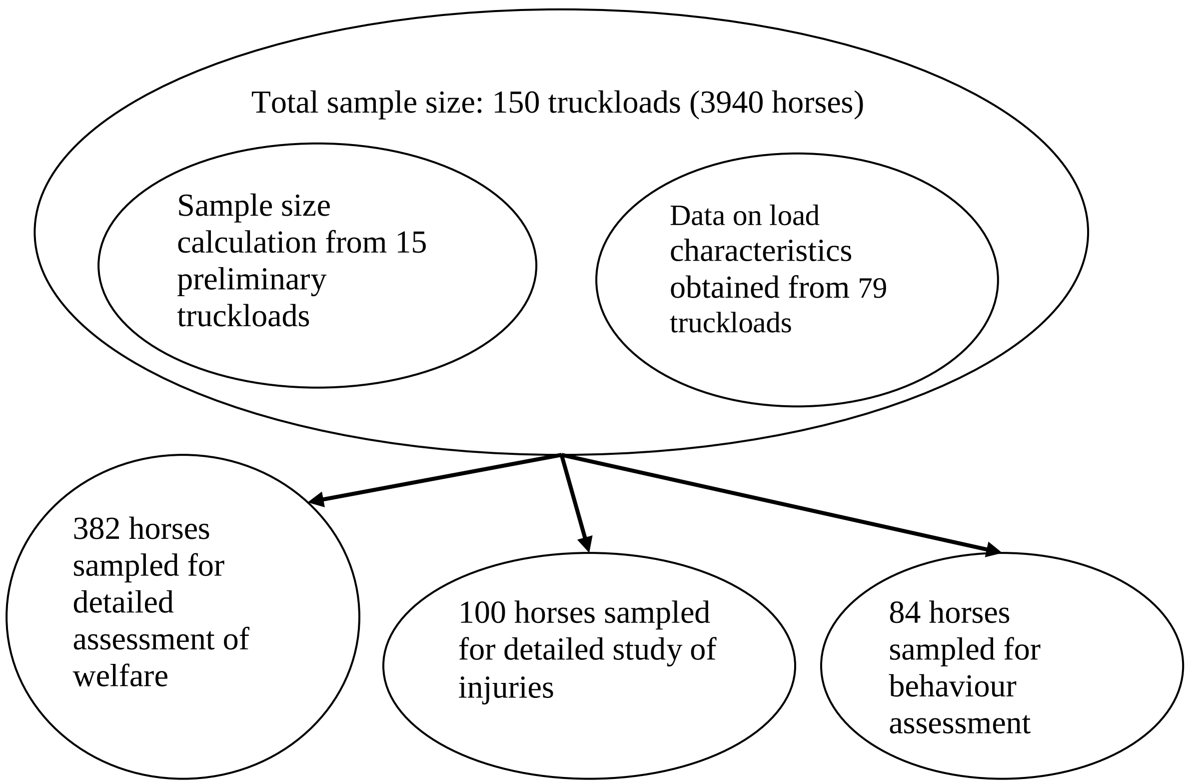


Figure 6.1: The sampling pattern performed in this study. Note: For a subset of 79 truckloads, detailed information regarding load characteristics was obtained from the slaughter plant. By convenience sampling, 382 horses were selected from 150 truckloads to study welfare issues in detail. Another 100 horses were studied in detail for assessment of injuries and 84 horses for behaviour assessment.

6.2.2.2. Detailed welfare assessment on a subset of horses

Once the horses had entered the lairage area, two to three horses were randomly selected (using the convenience sampling criteria that they possessed identifiable coat markings that could be used as a means for subsequent identification) from each truckload for detailed study (n=382). Sweating was assessed by observing the presence of wetness on the surface of the horse, and respiration rate was measured by counting the number of movements of the nostrils or flank region in one minute. General behaviour was categorised as 0= alert, 1=apathetic, 2= severely depressed and 3=agitated/excited (adapted from Burn *et al.*, (2010a, b) and Pritchard *et al.*, (2005)). Bite injuries were observed by identifying visible teeth marks in the skin.

Clinical observations

Lameness was assessed using the following categories: 0= no detectable abnormality , 1=noticeable defect in walking , 2=weight bearing on all limbs while walking, but visible or evident difficulty in walking , 3=able to stand up and walk, but foot raised so that the toe was touching the ground while standing , 4=non-weight bearing lameness and 5=recumbent . Skin temperature was measured with an infrared thermometer (TPI 381® Non-Contact Thermometer, Test products International, Ltd. 342 Bronte St. South, Unit 9, Milton, Ontario L9T 5B7) held at approximately 5 metres from the horse and directed towards the neck, flank and gluteal regions.

Blood measurements

At the point of slaughter (during exsanguination), blood samples from the previously selected 382 horses were collected into vacutainer tubes with lithium heparin anticoagulant. Measurement of lactate and glucose concentration in the whole blood was performed immediately. Blood glucose concentration was measured using an Alpha TRACK® blood glucose monitoring meter (Abbot Laboratories, Borth Chicago, IL 60064 USA) validated for use in horses (Hackett and McCue, 2010). Blood lactate concentration was measured using Lactate Pro LT-1710® (Arkray, Inc, 57 Nishi Aketa-cho, Higashi-Kuji, Minami-ku, Kyoto, Japan) which had been validated for veterinary use (Thorneloe *et al.*, 2007).

At the end of each working day, the blood samples were centrifuged for 20 minutes at 3000 revolutions per minute and the plasma extracted. Plasma osmolality was measured using an osmometer (Advanced Instruments, Inc., Norwood, Massachusetts, 02062, USA). Plasma total protein concentration was measured using a temperature compensated hand-held refractometer (Reichert®, Reichert, Inc. 3362 Walden Avenue, Depew, NY 14043 USA) with a conversion chart for equine plasma (Sutton, 1976). Blood glucose and lactate concentration, plasma total protein concentration and plasma osmolality values were compared with the normal clinical range for horses provided by Kaneko *et al.* (1997).

6.2.2.3. Specific assessment for injuries

As the character, size and severity of injuries could not be studied in detail for all 3940 horses from the 150 truckloads; 100 horses were subjected to detailed assessment for injuries. Two to 3 horses selected by convenience sampling from 40 truckloads, which were a subset of the 150 truckloads, were used for detailed assessment. To assess both visible injuries and those not visible to the naked eye, digital infrared thermographic imaging was undertaken ante-mortem and carcass bruising assessment was undertaken post-mortem.

Visual assessment of injuries

Injuries were assessed using visual assessment, by marking the location and size of the injury on a horse outline sketch – representing left and right side of the animal (Figure 6.2).

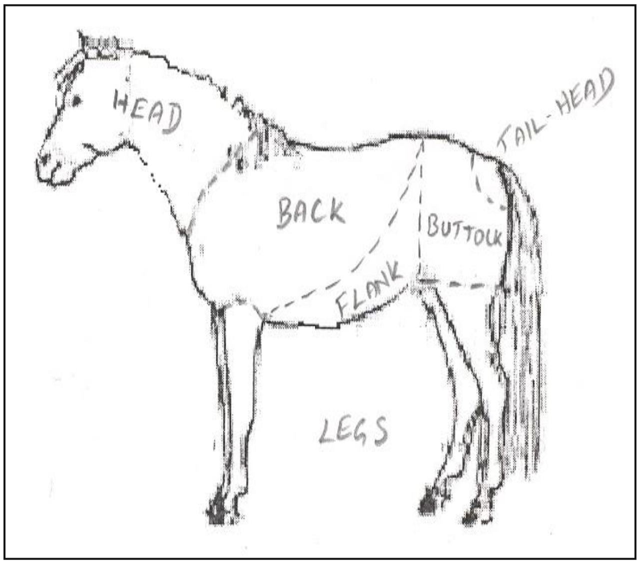


Figure 6.2: Outline sketch of the horse with demarcation of regions used for the assessment of injuries. The body of the horse was divided into regions of head, back, flank, buttock, tail head and legs. Two of these sketches were used for the assessment of a single horse, one of them to identify lesions on the left side of the body and another one on the right side.

The character of the injury was classified as follows: non bleeding open wound (0), bleeding wound (1), fibrin or dried exudates (2), infected pus (3), granulation wound (4) and scar (5). Severity of the injury was assessed by the layers of tissues involved (wound depth) (Hollander *et al.*, 1995) and classified as superficial wounds involving skin (1), subcutaneous tissues (2), muscle (3), tendon (4) and bone (5). The size of injury was categorised as small (≤1 cm^2 or 1cm x 1 cm or diameter 1.1 cm) medium (1.1 to 10 cm^2 or 3.3 cm x 3.3 cm or 1 x 10 cm or >1.1 to 3.5 cm diameter), large (10.1-20 cm^2 or 4.5 cm x 4.5 cm or 2 x 10 cm or 3 x 6.5 cm or diameter >3.5 to 5 cm) very large (20.1-100 cm^2 or 10 cm x 10 cm or 2 cm x 50 cm or 3 cm x 33 cm or 4 cm x 25 cm or diameter >5 cm to 11 cm) and extensive (>100 cm^2). Presence or absence of swelling was also noted.

Assessment of injuries using digital infrared thermography (DT)

Immediately before horses were moved into the stunning pen, DT images were obtained for 100 horses. Multiple DT images covering the whole region of the horse's body were obtained from each of the 100 horses from an elevated platform at a distance of 5 metres to use as a diagnostic test to detect bruising. After stunning, carcasses of the same 100 horses were examined for bruising and presence of bruising was marked using anatomical landmarks on two horse outline diagrams, one representing the left side of the animal and another the right side (standard test for bruising detection). While obtaining the images, the camera was set in such a manner that any area of the skin surface of the horse which had a higher temperature than the preset threshold temperature (determined using the method described in Chapter 5) appeared as a region of visibly increased thermal emission on the camera view screen. DT images were downloaded to a computer for detailed assessment using ThermaCAM Researcher software (FLIR Systems, Inc. 27700 SW Parkway Ave, Wilsonville OR 97070, USA). The emissivity was set at 0.96 (MacPhee, 2008) for all DT images.

Regions of increased thermal emission (red patches) identified from DT images were marked on two horse outline diagrams (to represent left and right side of the horse's body) so that comparisons could be made with the two similar horse diagrams from the carcass assessment of bruising (Figure 6.6). For each animal, if any one bruise on the carcass was identified on the DT image as a region of increased thermal emission on the same location (using anatomical landmarks), then that animal was considered as a true positive.

Assessment of bruising in carcass

After slaughter and skin removal, but before trimming, bruising was assessed on the carcass of each of the above selected horses (n=100) using an adaptation of the Australian Carcass Bruise Scoring System (Anderson and Horder, 1979). The size of the bruise was categorised as small (≤1 cm^2 or 1x1 cm or diameter 1.1 cm), medium (1.1 to 10 cm^2 or 3.3 cm x 3.3 cm or 1 cm x 10 cm or >1.1 to 3.5 cm diameter), large (10.1-20 cm^2 or 4.5 cm x 4.5 cm or 2 cm x 10 cm or 3 cm x 6.5 cm or diameter >3.5 cm to 5 cm), very large (20.1-100 cm^2 or 10 cm x 10 cm or 2 cm x 50 cm or 3 cm x 33 cm or 4 cm x 25 cm or diameter >5 to 11 cm) and extensive (>100 cm^2). The locations of the bruising were also recorded similarly to that of injury assessment, by drawing the location and size of bruising in a horse outline sketch.

Comparison of the regions of increased thermal emission (red patches) in DT images was undertaken with carcass bruising and visual assessment of injuries at the individual animal level (details in Section 6.2.3).

6.2.2.4. Behaviour assessment in lairage

From a subset of 29 truckloads of horses, 84 horses were observed for specific behaviours as illustrated in Table 6.1. Three horses from a single pen were selected per truckload by convenience sampling while the horses were in the lairage and were observed by direct observation continuously and simultaneously for 8 to 27 minutes, five hours after unloading. A handheld behaviour recorder (Psion organiser, Model LZ64, Psion plc, London, UK) pre-programmed using Observer software Version 3.0 (Noldus Information Technology, Wageningen, The Netherlands) was used to measure the frequency, and duration of these specific behaviours. The frequency of event behaviours (kick and bite), and duration of state behaviours (eating and drinking) were recorded and then downloaded into Excel sheets. As truckloads arrived at different times of the day and several other observations had to be made immediately on arrival, this five hour delay after unloading was maintained for all behaviour observations. This type of behaviour sampling and continuous recording has been recommended when the experimental conditions are difficult (Martin and Bateson, 2007), as experienced in this study. Event behaviours recorded were frequency (measured in reciprocal units of time) of kicking, biting, being kicked and being bitten. The presence or absence of food in the pen during lairage and pen size in the lairage (large size, 8.48 x 4.24 metres or small size, 4.24 x 2.2 metres) was recorded. The two individual pens used for stallions were excluded from the behavioural observations.

Table 6.1: Behavioural observations made in the lairage and definition of behaviours observed.

Behaviour		**Definition**
Event behaviours	Kick (kicking another horse)	One or both hind feet in contact with another horse
	Kicked (kicked by another horse)	Received contact from one or both hind feet from another horse
	Bite (biting another horse)	Teeth used to grasp skin of another horse, ears pinned back and lips retracted
	Bitten (bitten by another horse)	Skin grasped by the teeth of another horse
State behaviours	Eating	Prehension of food from the floor or hay net and chewing continuously
	Drinking	Horse’s lips touching the water in pan and showing swallowing reflex

6.2.3. Statistical analyses

Data were entered in Epidata (EpiData Association 2000-2012;http://www.epidata.dk/index.htm) and transferred to Stata 12.1 statistical software for analyses (StataCorp, 4905 Lakeway, College Station, Texas 77845 USA). Data analysis was performed in four parts. In the first part, descriptive statistics were performed on welfare outcomes to understand the prevalence of injuries, debility, pre-existing conditions and non-ambulatory condition. Summaries of physiological variables (skin temperature, respiration rate, blood lactate concentration, blood glucose concentration, plasma total protein and plasma osmolality) were explored to determine whether they were within the normal clinical range for horses, and the percentages of horses that differed from the normal ranges were calculated. In addition, descriptive statistics of various risk factors such as load characteristics, journey duration, and hours of lairage were calculated. As the data were not normally distributed, comparisons between summer and winter periods for respiration rate and skin temperature were undertaken using Wilcoxon rank sum tests. Two-sample test of proportions was used for comparing differences between the prevalence of injuries in horses from Canada and the USA. Correlations between the welfare outcomes, number of horses injured in each truckload with that of number of horses with swellings around the eye and number of horses having a body condition score less than 3 in each truckload were also performed.

The second stage of the analysis involved building statistical models to find associations between welfare issues and risk factors. Injuries, blood glucose concentration, blood lactate concentration, plasma osmolality and plasma total protein concentration were the outcome variables for which models were built with biologically plausible predictors as independent variables to understand associations. The number of horses with visible injuries in each truckload followed a count data pattern and hence a negative binomial multivariable regression model was used. In this model, the total number of animals in each load was considered as an offset (compensating for the changing number of horses in each truckload). The predictors in this model were origin of horses, journey duration (in hours), stocking density during transport and number of horses that had a body condition score less than 3. The stocking density did not have a significant effect on the prevalence of injuries as evaluated by including them in the model and then by removing them, evaluating the change in coefficients and also by examining the significance level. Hence stocking density was dropped from the final model. Evaluation of the model was undertaken by goodness of fit test and diagnostic plots of residuals (Anscombe residuals Vs predicted count and Anscombe residuals Vs Cooks distance) (Dohoo *et al.*, 2009).

Statistical models were built to determine associations between physiological measures (blood glucose concentration, blood lactate concentration plasma osmolality and plasma total protein concentration) and biologically plausible predictors such as whether the horses were lairaged or not, season (summer, winter) and declared duration of the final stage of journey to the slaughter plant. As the physiological measures were at the animal level and predictors were at the truckload level, a linear mixed model with truckload as a random effect was used to evaluate these associations (Dohoo *et al.*, 2009). The blood lactate concentration variable had to be log transformed to meet the model assumptions. The final model was evaluated for normality (by plotting the residuals in the form of a histogram and by using the Shapiro-Wilk test for normality), homoscedasticity (by plotting the standardised residuals against the predicted values) and linearity (graphical assessment- lowess smooth plots between outcome and continuous predictors). Outliers were also evaluated for any undue influence and leverage on the model. Intra-class correlation coefficients were examined to evaluate the level of correlation between horses within truck loads. Post-estimation pair-wise comparisons were performed whenever necessary (effect of winter and summer on blood lactate concentration – pair-wise comparison).

The third part of the analyses consisted of a summary of event behaviours (kick or kicked and bite or bitten) and state behaviours (duration of time spent eating and drinking). The percentage of horses that showed these behaviours was also summarized. As behaviours, such as kicking and biting could lead to injury, these behaviours themselves were also used as outcome variables. The association between these event behaviours and two predictors; availability of food in the pen and size of the pen, were examined by regression analysis. Frequencies of event behaviours were modelled as count data (mixed effect Poisson model with pen as the random effect).

The final part of the analysis was to determine the agreement of proportions between injuries detected by the DT as abnormal red patches with that of visual injuries and carcass bruising. Agreement was evaluated at the animal level, whereas previously in chapter 5 agreements were tested at region level (left and right side of the animal). The overall proportion of agreement between visually detected injuries and abnormal patches detected by DT was compared using two-sample tests of proportions. Similar comparison was made between carcass bruising and abnormal patches detected by DT. Dohoo *et al.* 2009 suggested that while comparing two diagnostic tests, neither one being a gold standard test, kappa statistics can be used to evaluate the agreement of proportions. However, before quantifying kappa statistics, assessment had to be performed to determine whether the two tests are classifying the same proportion of horses or lesions as positive. A McNemar test was undertaken to assess if DT as a diagnostic tool detected the same proportion of positive animals as visually detected injuries and carcass bruising. Then a kappa statistic was computed to determine the quality of agreement.

6.3. RESULTS

6.3.1. Load characteristics

Most of the horses (130 truckloads) that arrived at the slaughter plant in Québec (Canada) were from the USA. Only a small percentage (20 truckloads) came from within Canada, all of which from Ontario. The slaughter horses from the USA started the final stage of their journey to the slaughter plant from one of five auction markets located in Ohio, Iowa, Pennsylvania, Kentucky and Indiana. Table 6.2 provides the summary of load characteristics. The average carcass yield percentage (mean and standard deviation) was 59 (±1.4) % which was calculated from 79 truckloads of data and was obtained from the slaughter plant (subset of the 150 truckloads). Out of the 382 horses sampled from the 150 truckloads for detailed observation, 66% were mares, 33% geldings, and 1% stallions.

Table 6.2: Summary of load characteristics: total weight of horses per load, average horse weight in each load, and stocking density during transport for the 150 truckloads sampled.

Load characteristics	Truck loads (N)	Minimum	Q_1	Median	Q_3	Maximum
Number of horses in each load	150	4	27	30	30	39
Total weight of horses in each load (kg)	77	1330	11,516	12,759	13,368	16,174
Average live weight in each load (kg/horse)†	59	245	418	431	455	575
Assumed stocking density (kg/m^2)	62	36	326	349	362	439

† Live weight obtained from slaughter plant records

6.3.2. Injuries, fitness and non-ambulatory conditions

Horses (n= 3940 horses) from 150 truckloads were examined for the presence of any visible injuries. When compared by the two-sample test of proportions, the prevalence of injuries was significantly higher in horses transported from the USA (12.8%) than in horses transported from Canada (4.2%) ($P<0.001$) (Table 6.3). When the unconditional association between number of horses injured in each truckload and origin of the horses (Canada or the USA) were examined, there was a significant association. Season (summer or winter) did not have any significant association with number of animals injured per truckload. Among the various states (Ohio, Iowa, Pennsylvania, Kentucky and Indiana) from which horses from the USA were transported, the prevalence of injuries ranged from 12 to 16.4% (Table 6.4). There was a significant positive correlation between number of horses injured in each truckload and number of horses with swellings around the eyes ($P<0.001$). The overall mean prevalence of horses with head injuries was 4.6% (confidence interval of 4 to 5.3%) (183 horses had head injuries when 3940 horses were observed). The overall mean prevalence of horses with swelling around the eyes was 1.1 % (confidence interval of 0.8 to 1.4%).

When compared by the two-sample test of proportions, the prevalence of horses with pre-existing conditions was significantly higher in horses transported from Canada (6.8%) than in horses transported from the USA (1.4%) ($P<0.001$) (Table 6.3). The common pre-existing conditions observed were severely swollen joints (chronic arthritis) and granulation wounds in the limbs. However, there was no significant difference in the prevalence of horses having a body condition less than 3 between horses arriving from Canada and the USA. When the proportion of horses having a body condition score less than 3 in each truckload was compared with the proportion of horses injured in each truckload, there was a positive, but not statistically significant correlation ($\rho = 0.14$).

All of the horses that arrived in a non-ambulatory condition were observed in the truckloads that arrived at the slaughter plant from the USA. A total of six horses were non-ambulatory, one horse each from six truckloads. Four of these truckloads were from Ohio, whereas the other two were from Indiana. The average number of horses loaded in these consignments that contained a non-ambulatory horse was 29. The average journey duration of transport for these truckloads was 32 hours. The average number of horses with injures in the truckloads that had non-ambulatory horses was five.

Out of 345 animals assessed for lameness only four animals were visibly lame. One of the horses had only minor lameness, whereas the other three had one limb raised from the ground, which indicated serious lameness.

Table 6.3: Prevalence of injuries, body condition score less than 3, pre-existing conditions, and non-ambulatory conditions in horses that originated from the USA and Canada. Prevalence was calculated after observing 3940 horses from 150 truck loads.

Country of origin	Number of horses observed (n)	Prevalence of horses with injuries % (CI)	Prevalence of horses with body condition <3 % (CI)	Prevalence of pre-existing conditions % (CI)	Prevalence of non-ambulatory horses % (CI)
USA	3750	12.8 (11.8-13.9)[a]	4.6 (3.9 -5.3)[a]	1.4 (1.1- 1.8)[a]	0.16 (0.05-0.35)
Canada	190	4.2 (1.8- 8.1)[b]	5.3 (2.6- 9.5)[a]	6.8 (3.7-11.4)[b]	0

CI-confidence interval

[a,b] Same alphabetic superscript along vertical column indicates no significant difference between the USA and Canada and different alphabetic superscript indicates significant difference.

Table 6.4: Prevalence of injuries, head injuries, and swellings around eyes tabulated according to province/state from which the horses started their journey and journey duration (declared documentary evidence for journey times)

Province/State	Journey duration in hours (mean ±sd)	No. of horses observed	No. of truckloads	Prevalence of horses with injuries % (CI)	Prevalence of horses with head injuries % (CI)	Prevalence of horses with swelling around eyes % (CI)
Ontario	11.10 ±1.02	190	20	2.6 (0.9-6.10)	0	0.5 (0.01-2.9)
Pennsylvania	17.17±5.40	1205	41	12.0 (10.2-14.2)	2.6 (1.7-3.7)	0.8 (0.4-1.5)
Ohio	29.19 ±4.45	1494	55	13.2 (11.5-15.2)	2.7 (1.9-3.6)	1.2 (0.7-1.9)
Iowa	34.23 ±2.17	688	22	12.5 (10.0-15.2)	3.0 (1.9-4.7)	1.0 (0.4-2.1)
Indiana	34.50 ±2.00	235	8	14.9 (10.4-20.7)	4.7 (2.3-8.4)	1.3 (0.3-3.7)
Kentucky	36.00 ±0	128	4	16.4 (10.2-25.1)	1.5 (0.2-5.6)	1.5 (0.2-5.6)

CI=confidence interval, sd=Standard deviation

6.3.3. Injury characteristics

Of the 100 horses from 40 truckloads studied in detail for injuries, 33 had injuries (33%) by visual assessment, 72 had bruising (72%) by carcass assessment, and 48 had abnormal regions of thermal emission (48%) detected by DT. Each injured horse exhibited single injuries or multiple injuries in different regions. Injuries by visual assessment, bruising by carcass assessment, and abnormal thermal emission patches by DT and the percentages of their distribution regarding location in the body are shown in Table 6.5 and Figure 6.3. Determination of severity of injuries in the 33% of affected horses with injuries by visual assessment showed that 27% of horses had superficial wounds and 6% had subcutaneous tissue injuries. Fifteen percent of injuries were accompanied by swelling and the other 85% did not have any swelling. Sixty four percent of injuries were non-bleeding and 36% were bleeding wounds. Thirty one per cent of injuries were small, 66% were of medium size and 3% were large. For bruising by carcass assessment, 48% of bruises were small, 46% were of medium size, and 6% were large. The prevalence of horses with bite injuries was 11%.

Table 6.5: Number of injuries at each predefined location in 100 horses (Figure 6.2) using three methods of assessment: (i) assessment of visible injuries, (ii) carcass assessment for bruising and (iii) by DT. Some horses had more than one injury.

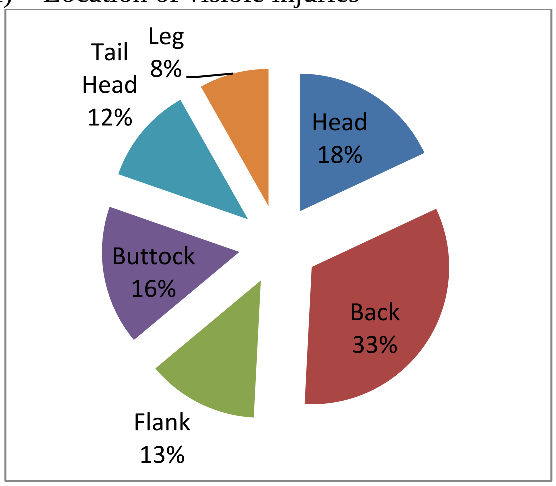
Injuries assessment		Location on body					
		Head	Back	Flank	Buttock	Tail-head	Leg
i)Visual		11	20	8	10	7	5
Severity[1]	Superficial wound	6	17	8	9	6	4
	Subcutaneous wound	5	3	0	1	1	1
Character[1]	Non-bleeding	6	20	8	9	7	5
	Bleeding	5	0	0	1	0	0
Size categories[1]	Small	0	0	0	9	6	4
	Medium	11	18	8	1	1	1
	Large	0	2	0	0	0	0
Swelling	Present	4	3	0	1	1	1
	Absent	7	17	8	9	6	4
ii) Bruising		18	19	4	24	4	41
Size categories[12]	Small	7	13	1	8	2	22
	Medium	11	6	2	12	2	17
	Large	0	0	1	4	0	2
iii)DT images (red patches)		11	14	3	15	12	18
Size categories[12]	Small	4	5	2	5	4	8
	Medium	7	8	0	8	8	9
	Large	0	1	1	2	0	1

[1] Only categories with types of injury observed are listed in the table. Some categories of injury were not observed.

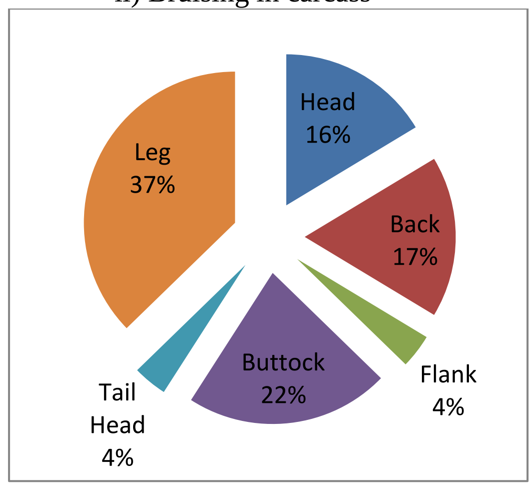
[2]these size categories were estimated because carcass bruising had to be assessed from a distance and abnormal patches from DT had to be obtained from images.

Out of the 11 head injuries that were visible (Table 6.5), five injuries involved subcutaneous tissue damage, were medium sized and bleeding. Four of them also had associated swelling. All the other six injuries were superficial, non-bleeding, medium sized and except for one, were without swelling. All of the twenty visible injuries on the back region were non-bleeding, most were superficial medium sized wounds, but two of them large sized. The eight visual injuries on the flank were superficial, non-bleeding and medium sized. Nine of the ten visual injuries on the buttock were superficial, non-bleeding and small. The remaining one buttock injury was subcutaneous, bleeding, and medium sized with swelling. Six of the seven injuries on the tail-head were non-bleeding, superficial, and small. The remaining one was a subcutaneous and medium sized wound with swelling. Four of the five visually identified injuries in the leg region were non-bleeding, superficial, and small. The remaining one was a subcutaneous, medium sized wound with swelling.

i) Location of visible injuries



ii) Bruising in carcass



iii) Abnormal areas of thermal emission detected by DT

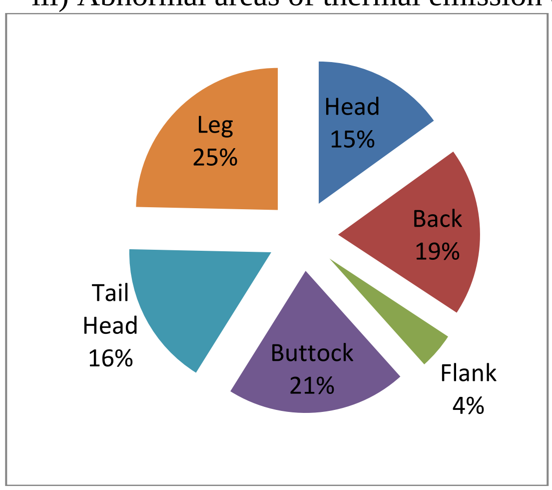


Figure 6.3: Pie diagrams showing the percentage distribution of all of the visible external injuries (i), carcass bruising (ii) and abnormal patches in DT (iii) according to location on the body of injured horses. These percentages were calculated from the subsample of 100 horses, out of which 33 had visible injuries by visual assessment, 72 carcasses had bruising and 48 of them had abnormal regions of thermal emission detected by DT. Injured horses had single or multiple injuries in multiple locations.

6.3.4. DT assessment of injuries

Out of the 100 horses for which DT were performed to assess injuries, 48% (confidence interval of 39% to 58%) of the horses showed abnormally high emission patterns in one or several regions of the body (Figure 6.4; a2 and Figure 6.5). The percentage of animals detected for bruising visually by examining the carcass was 72% (confidence interval of 63% to 80%). Regions of abnormal thermal emission were located on the leg, buttock, back region, tail-head, head, and flank in descending order of the percentage of abnormal area recorded (Figure 6.3, iii). At the animal level, when the proportion of agreement was tested by a two-sample test of proportions, agreement showed significant difference between visual assessment for injuries and DT abnormal emission. When a McNemar's test was done to evaluate the proportion of agreement, again there was no significant agreement between carcass bruising and DT abnormal emission indicating that the proportion of horses with lesions detected by the two methods differed significantly. The percentage of horses that had visual injuries (skin injuries) was 33% (confidence interval of 23% to 42%).

a 1: Horse without any abnormal emission **a 2: Horse with abnormal zones**

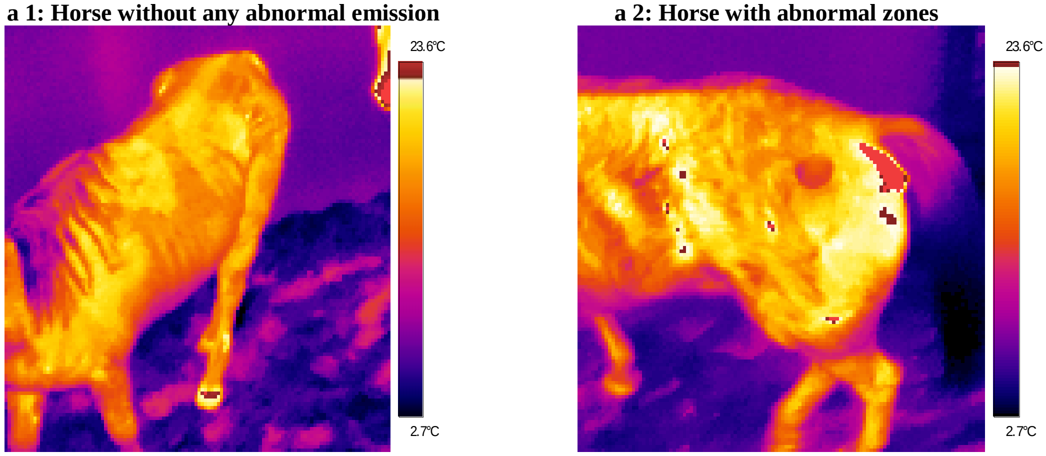


Figure 6.4: a1 and a2 are DT of two horses taken immediately after arrival at the slaughter plant. The horse on the left shows uniform distribution of skin temperature throughout its body, whereas the horse on the right shows uneven distribution of skin temperature which may indicate injury. For both of these DT, the infrared camera was preset in such way that if any part of the body was above 23.1ºC that area would appear as an abnormally high area of emission (red patches).

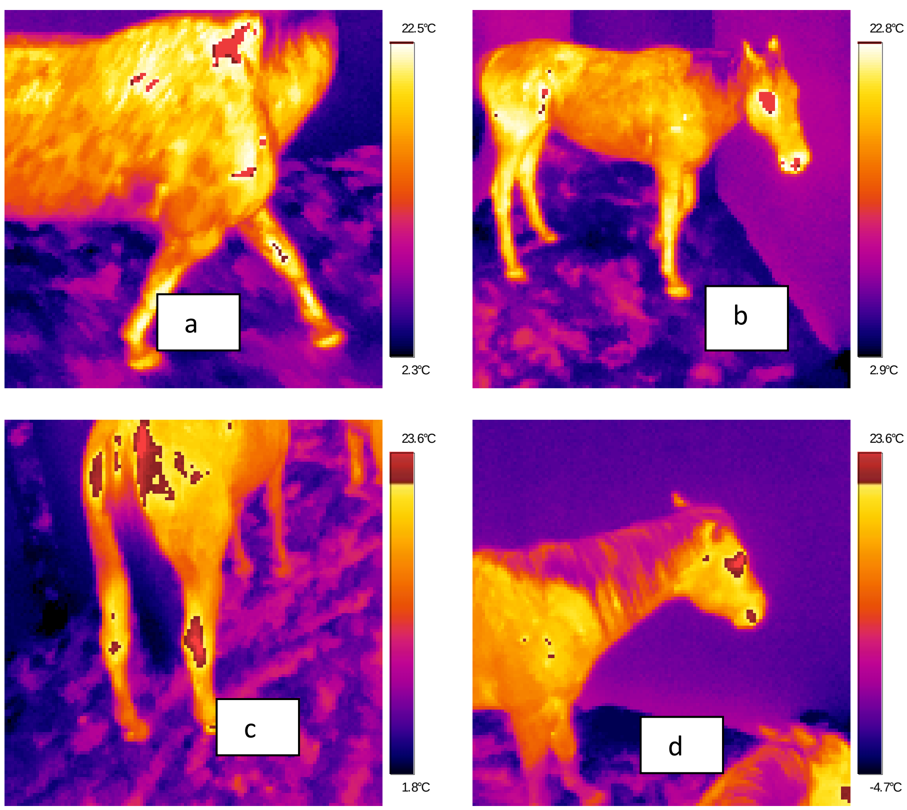


Figure 6.5: a, b, c, d was DT of four horses showing abnormally high zones of thermal emission on the body surface which are areas of surface skin temperature above the preset threshold temperature for that day.

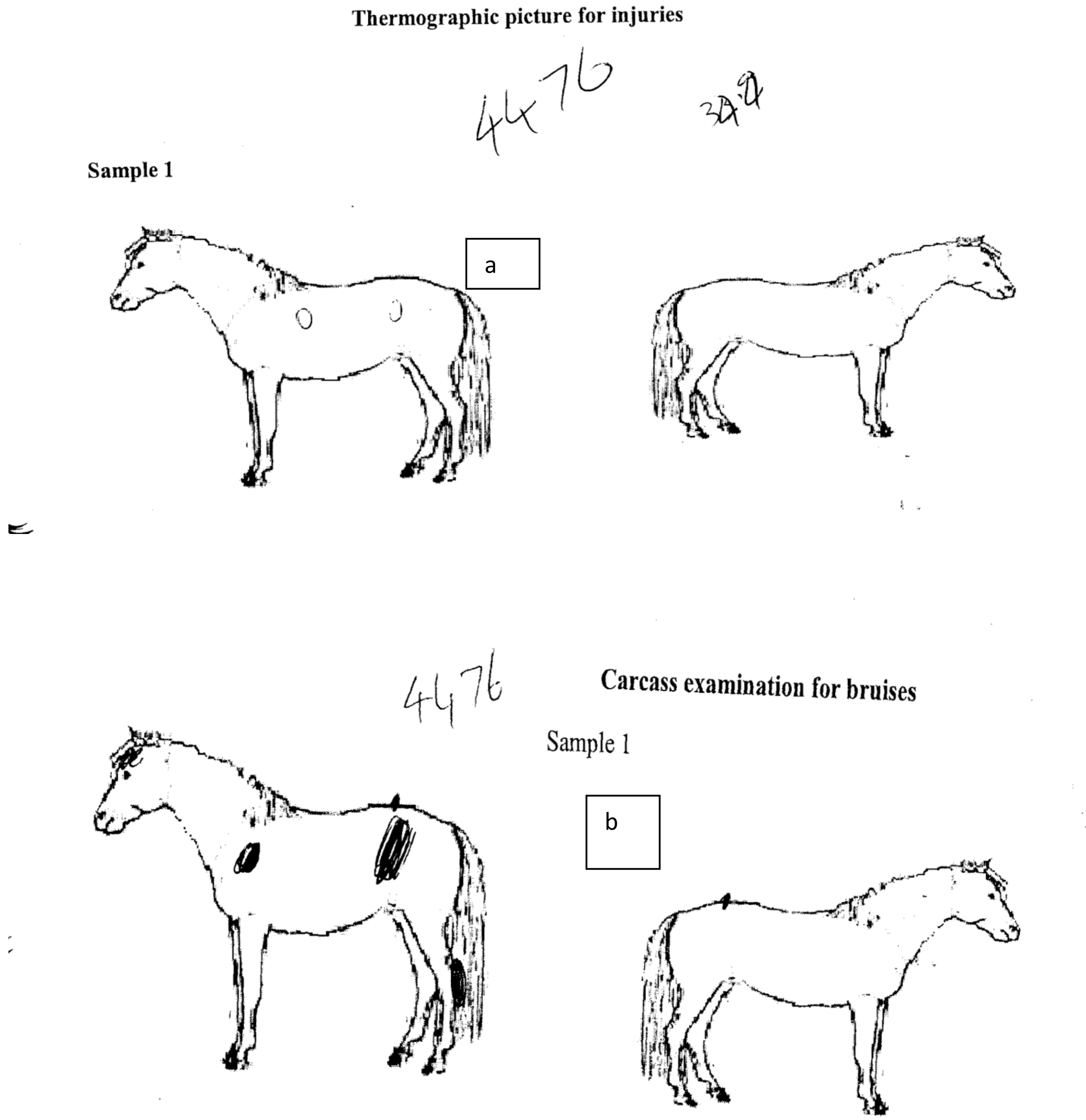


Figure 6.6: Comparison between assessment of bruising by abnormal zones of thermal emission by DT (a) and carcass examination (b) of the same horse. Picture (a) shows the DT abnormal patches noted on the left and right side of sample horse number 4476 and Picture (b) shows the bruising in the same area as the abnormal DT patches and also in additional regions.

6.3.5. Risk factors for injuries

Journey duration, proportion of horses with body condition less than 3 in each truckload, origin of horses (Canada or the USA) and stocking density were the predictors for which associations were examined using a negative binomial regression model with number of horses having external visual injuries in each truckload as the outcome variable. The number of horses in each truck load was included in the model as an offset; thereby the variation due to this variable on the outcome (number of horses with injuries) could be accounted for. Stocking density did not have a significant association with the number of horses injured in the truck. Journey duration (declared documentary evidence for journey time) and origin had a significant effect on the number of horses per truckload with injuries (Table 6.6). Figure 6.7 illustrates that when the journey duration increased, the number of horses with injuries per truckload increased significantly ($P<0.001$).

Table 6.6: Negative binomial model with number of horses in each truckload with an injury as the outcome and three predictors; journey duration, origin of journey, number of horses with body condition less than 3 (overall significance of the model was $P<0.001$). Parameter estimates, Standard error of the coefficient (SE) and significance level are tabulated.

Predictor		Coefficient (log-count of horses injured per truck)	SE	P value
Journey duration (hours)		0.02	0.007	0.005
Origin	Canada	Ref	n/a	n/a
	USA	1.01	0.375	0.007
Number of horses with body condition less than 3		0.62	0.035	0.078
Constant		-3.64	0.383	0.001

n/a-not applicable, Ref-reference level.

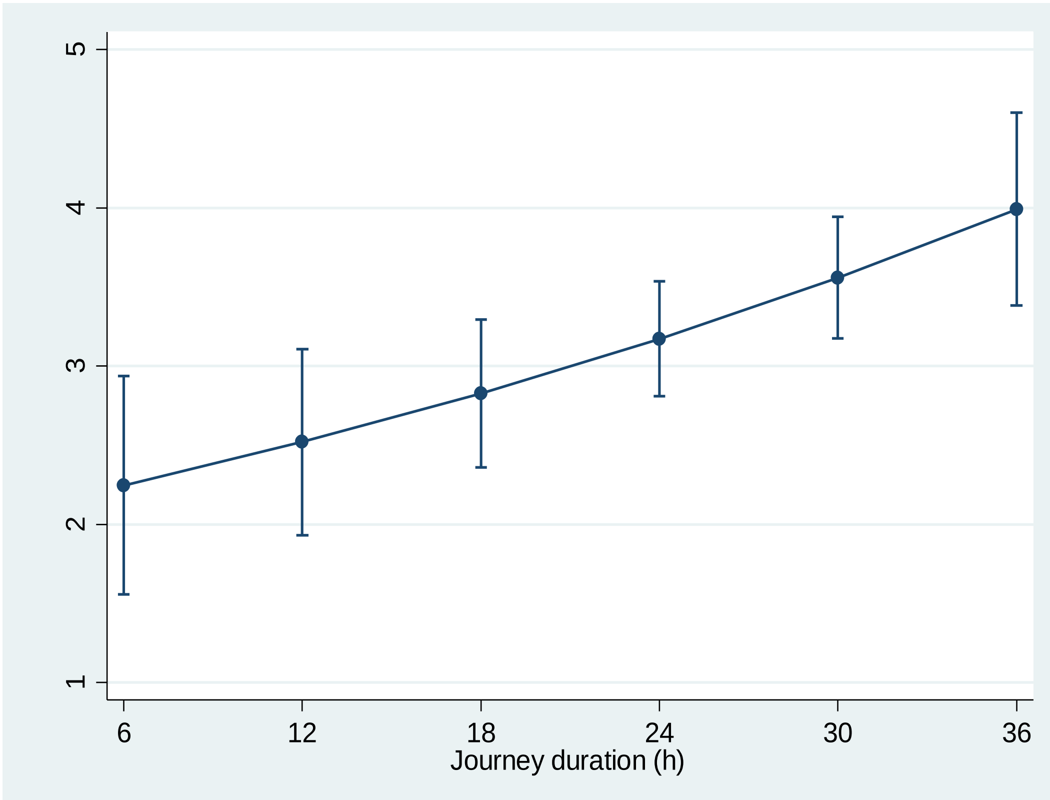


Figure 6.7: Number of horses with injuries (predicted) per truckload (loaded with an average number of horses) plotted against journey duration in hours. Confidence interval (95%) was calculated at 6, 12, 18, 24, 30 and 36 hours of journey duration. This graph was generated from the model described in Table 6.6.

6.3.6. Clinical assessments

When horses were examined for general attitude immediately after arrival at the slaughter plant, 96.6% of the horses were alert and the remaining 3.4% were apathetic, but none were severely depressed. When truckloads which had apathetic horses were examined, the journey duration ranged from 26 to 36 hours and all these consignments had injuries, particularly head injuries. Most of these journeys were from Ohio in the USA. Less than 1% of horses showed signs of sweating. Similarly, less than 1% of horses showed obvious signs of lameness. The body condition of horses arriving at the slaughter plant was generally good. However, approximately 5% of horses had a body condition score of less than 3 (Table 6.3). Prevalence of horses with a body condition score less than 3 was similar for horses from the USA and Canada.

6.3.7. Physiological measurements

The skin temperature as measured by infrared thermometer ranged from 5ºC to 33ºC. When compared using Wilcoxon rank sum tests, there was a significant reduction in skin temperature during winter (median 17.4ºC) compared to summer (median 28.3ºC) (P=0.001). Respiration rate ranged from 14 to 42 breaths per minute. There was a significantly higher respiration rate in winter (median of 20 breaths per minute) than in summer (median of 15 breaths per minute) (P=0.001). Table 6.7 summarizes the four physiological variables studied (blood glucose concentration, blood lactate concentration, plasma total protein concentration and plasma osmolality) according to place of origin and journey duration. Table 6.8 and Figure 6.8 summarise the normal clinical range for physiological variables for horses and percentage of horses with values below or above the normal clinical range for blood lactate concentration, blood glucose concentration, plasma total protein, and plasma osmolality.

Table 6.7: Median and range of the four physiological variables measured (blood glucose concentration, blood lactate concentration, plasma total protein and plasma osmolality) summarised according to the place of origin of consignment and the mean journey duration.

Province/State	Journey duration in hours (mean)	No. of horses observed (n)	Blood glucose concentration Median (range) (mmol/l)	Blood lactate concentration Median (range) (mmol/l)	Plasma total protein concentration Median (range) (g/l)	Plasma osmolality Median (range) (mmol/kg)
Ontario	11.10	53	5.8 (3.7-9.2)	5.1 (1.8-17.8)	81 (67-100)	297 (284-323)
Pennsylvania	17.17	134	6.6 (4.5-9.8)	4.5 (1.4-12.3)	80 (55-115)	295 (272-320)
Ohio	29.19	67	6.4 (4.3-9.9)	4.9 (1.0-12.7)	84 (57-104)	295 (280-318)
Iowa	34.23	93	6.6 (4.1-9.9)	5.3 (2.0-10.9)	85 (70-105)	295 (275-321)
Indiana	34.50	10	6.9 (5.2-8.1)	5.8 (1.6- 9.8	82 (64-92)	300 (289-316)
Kentucky	36.00	19	6.0 (4.8-8.2)	4.9 (1.6- 9.1)	84 (76-112)	295 (279-314)

Table 6.8: Percentage of horses within, below, and above normal clinical range for the blood variables measured post slaughter at exsanguination.

Blood Variables	Normal clinical range (Kaneko *et al.*, 1997)	n	Horses within normal range (%)	Horses below normal range (%)	Horses above normal range (%)
Blood lactate concentration (mmol/l)	1.1-1.7	376	1.6	0.3	98.1
Blood glucose concentration (mmol/l)	4.1-6.3	375	49.2	0.3	50.5
Plasma total protein concentration (g/l)	52-79	360	26.6	0	73.4
Plasma osmolality(mmol/kg)	270-300	359	72.4	0	27.6

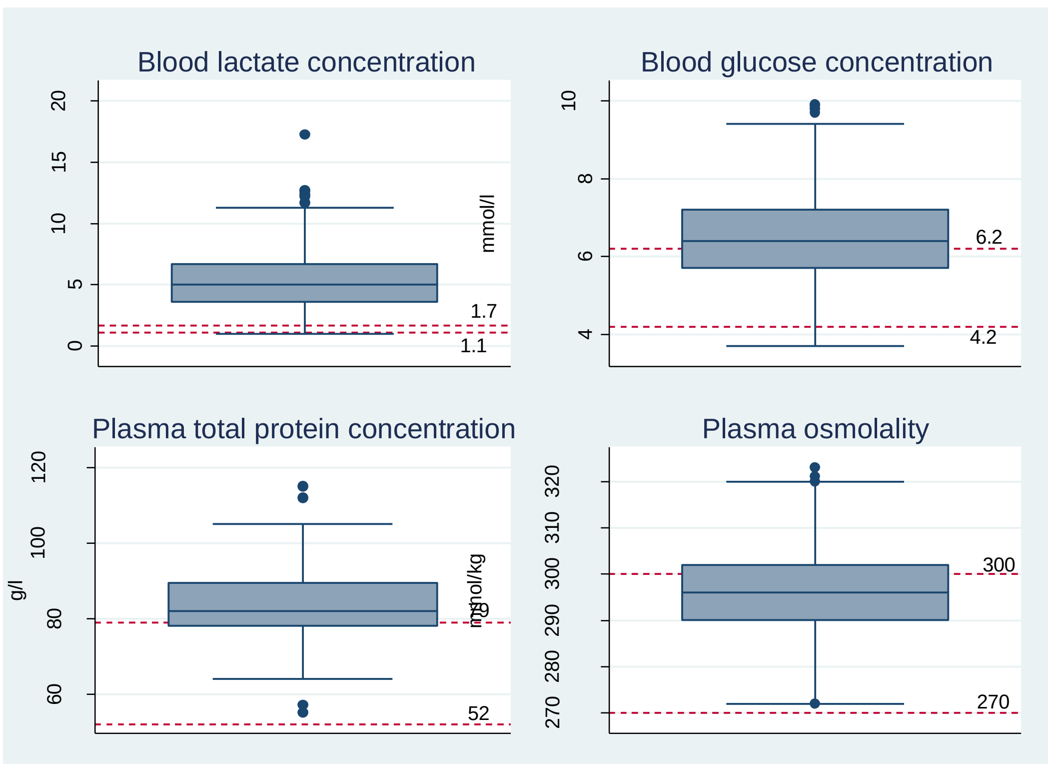


Figure 6.8: Box plot showing the blood concentration of lactate and glucose, plasma total protein and plasma osmolality. The boxes indicate the upper and lower quartiles with the median as the line in-between. The whiskers indicate the maximum and minimum values. • indicates an outlier, defined as those values which are 2.5 times the interquartile range from the median value. The dotted horizontal lines indicate the normal clinical range.

6.3.7.1. Associations between blood lactate concentration and risk factors

Blood lactate concentration had an interaction effect with season (summer or winter) and lairage (yes or no). Out of the 150 truckloads of horses observed, 79 truckloads did not undergo any lairage before slaughter, whereas, another 65 truckloads stayed in the lairage for at least one night (for 6 loads observation not possible). In the summer, provision of lairage time for horses after transport increased the blood lactate concentration (not statistically significant), whereas in the winter, provision of lairage time significantly decreased the blood lactate (Table 6.9, Figure 6.9). The journey duration to slaughter did not have any significant effect on the blood lactate concentration. The intra-class clustering was 12% which denotes the correlation between horses in consignments.

Table 6.9: Parameter estimates with standard error of the coefficient (SE) and P value for final model of log transformed blood lactate concentration. The predictors were journey duration in hours, lairaged or not lairaged (binary) and season (summer or winter) (overall significance of P<0.001 for the model, Intra-class correlation coefficient of 14% which is the variation explained by the consignment effect).

Predictor	Levels	Blood lactate concentration coefficient (log)	SE	P value
Journey duration (h)		0.001	0.003	0.915
Lairage *x* Season	No lairage, Summer	Reference level	n/a	<0.001†
	No lairage, Winter	-0.169	0.077	0.029
	Lairaged, Summer	0.110	0.068	0.106
	Lairaged, Winter	-0.377	0.069	<0.001
Estimate for constant/intercept		1.654	0.082	<0.001

n/a=not applicable, *x*=interaction, † Overall P value for "Lairage *x* Season"

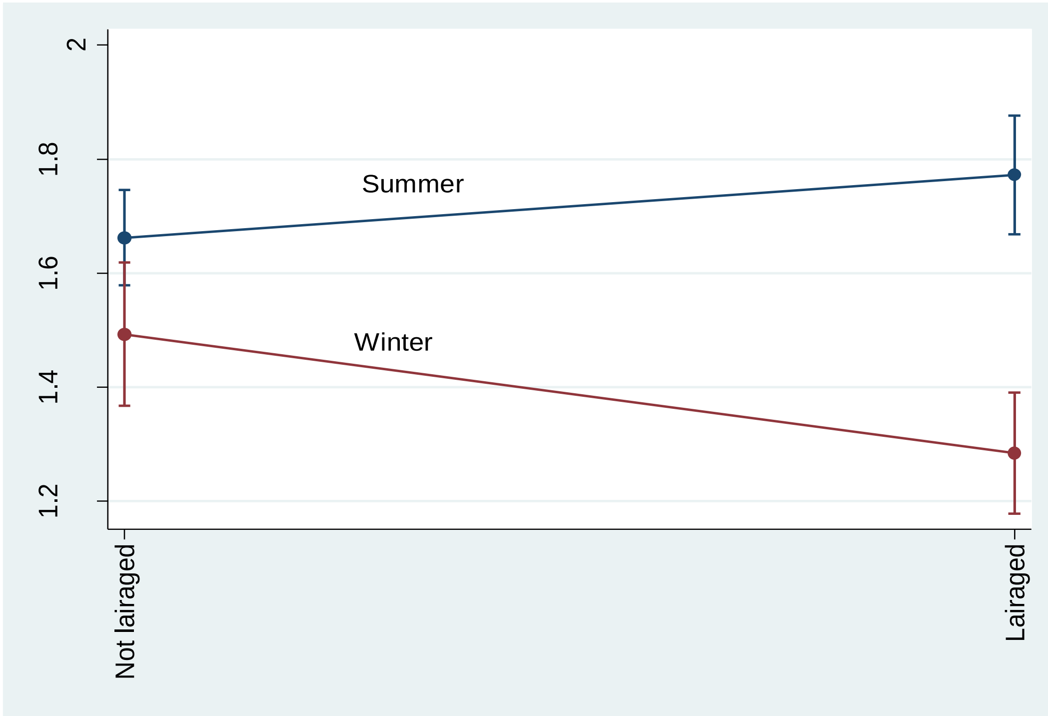


Figure 6.9: Predicted marginal estimates and confidence interval of blood lactate concentration (log scale) calculated from Table 6.9. Season (summer or winter) had an interaction effect with provision of lairage.

6.3.7.2. Associations between blood glucose concentration and risk factors

As illustrated in Table 6.10, provision of lairage significantly reduced the blood glucose concentration in horses. Winter season significantly increased the blood glucose concentration of horses. Place of origin and journey duration did not have a significant effect on the blood glucose concentration. The intra-class clustering was 18% which denotes the correlation between horses in the same consignment.

Table 6.10: Linear mixed model with blood glucose concentration as the outcome and province/state, journey duration, lairage (yes or no) and season (summer and winter) as predictors. Overall significance of the model was P=0.001 and intra-class correlation coefficient of 20% which is the variation explained by the consignment effect).

Predictor	Blood glucose concentration coefficient	SE	P value
Province/State			
Ontario	Ref	Ref	0.15†
Ohio	0.23	0.36	
Iowa	0.33	0.44	
Pennsylvania	0.58	0.23	
Kentucky	-0.02	0.61	
Indiana	0.51	0.49	
Journey duration	0.02	0.02	0.21
Lairage -No	Ref	n/a	n/a
Lairage- Yes	-0.52	0.12	0.001
Summer	Ref	n/a	n/a
Winter	0.36	0.12	0.01
Constant	5.71	0.25	0.001

Ref=reference level, n/a=not applicable, †Overall P value for categorical variable

6.3.7.3. Associations between plasma osmolality and risk factors

As illustrated in Table 6.11, province or State of origin was the only factor significantly associated with plasma osmolality. In comparison to the reference estimate (province of Ontario), most horses which arrived from four states of USA had higher plasma osmolality, except horses from Pennsylvania which had lower plasma osmolality than horses from Ontario, Canada. The intra-class clustering was 27% which indicates a high degree of correlation between horses in the same consignment.

Table 6.11: Linear mixed model with plasma osmolality as the outcome variable and province/state, journey duration, lairage (yes or no) and season (summer or winter) as predictors (Overall significance of the model was P=0.07 and intra-class correlation coefficient was 27% which is the variation explained by the consignment effect)

Predictor		Plasma osmolality- coefficient	SE	P value
Province/State				
Ontario		Ref	n/a	0.03†
Ohio		4.0	3.47	
Iowa		4.46	4.21	
Pennsylvania		-1.49	2.26	
Kentucky		6.09	5.77	
Indiana		10.33	4.72	
Journey duration		0.28	0.15	0.072
Lairage	No	Ref	n/a	n/a
	Yes	-1.7	1.21	0.14
Summer		Ref	n/a	n/a
Winter		-0.69	1.25	0.57
Constant		5.71	0.25	0.001

Ref=reference level, n/a=not applicable, †Overall P value for categorical variable

6.3.7.4. Associations between plasma total protein concentration and risk factors

As illustrated in Table 6.12, journey duration and season (summer or winter) had a significant effect on the plasma total protein concentration. As the journey duration increased, the plasma total protein concentration also increased (Figure 6.10). In comparison to winter, the total plasma protein concentration was significantly higher in summer.

Table 6.12: Parameter estimates with standard error of the coefficient (SE) and P value for final model of plasma total protein concentration with journey duration in hours, lairaged or not lairaged (binary) and season (summer or winter) as predictors (overall significance of P=<0.001 for the model and intra-class correlation coefficient of 16% which is the variation explained by the consignment effect).

Predictor		Plasma total protein concentration coefficient	SE	P value
Journey duration		0.25	0.05	0.0001
Lairage	No	Ref	n/a	n/a
	Yes	-0.46	1.01	0.64
Summer		Ref	n/a	n/a
Winter		-5.37	1.04	0.0001
Estimate for constant/intercept		79.04	1.55	0.0001

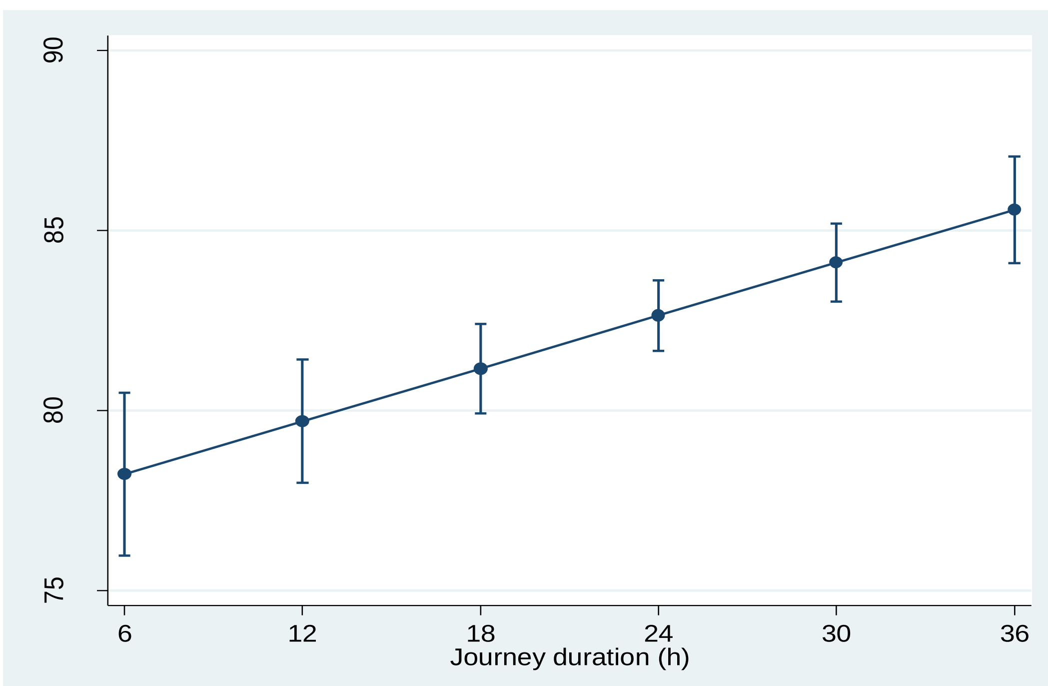


Figure 6.10: Parameter estimate of plasma total protein concentration with 95% confidence interval, when journey duration was plotted at 6, 12, 18, 24, 30 and 36 hours. Estimates obtained from the model in Table 6.12.

6.3.8. Behavioural observation in lairage

Table 6.13 illustrates the summary frequency of the event behaviours (kick and bite) obtained by observing 84 horses (from 29 truckloads housed in 12 pens of small and big sizes). Frequencies were calculated in reciprocal units of time in minutes (Number of units of behaviour per minute). The percentage of the observation period during which horses were observed standing and eating ranged from 0 to 100%. The percentage of the observation period during which horses were observed standing and drinking ranged from 0 to 84%. The effect of two predictors of interest during lairage which could have had an effect on kicking and biting behaviour, the size of the pens (8 small and 4 big) and presence or absence of food were examined. Food availability and pen size did not have any effect on any of the event behaviours as elicited in Table 6.14 and Table 6.15.

Table 6.13: Summary frequency of event behaviours of horses observed in lairage for a range of 8 to 27 minutes (n=84 horses). Frequencies of behaviours are expressed per hour.

Event Behaviour	**Percentage of horses exhibiting the behaviour (n=84 horses)**	**Frequency of behaviour (no. of events per horse per hour)**				
		Min	**Q_1**	**Median**	**Q_3**	**Max**
Kick	21	0	0	0	0	9
Kicked	26	0	0	0	3	38
Bite	29	0	0	0	3	30
Bitten	11	0	0	0	0	18

Table 6.14: Poisson model with frequency of kicking as the outcome and pen size and food availability as predictors (overall significance of the model was P=0.47).

Predictors	Coefficient of frequency of kicking	Standard error	P value
Pen size (small)	Ref		
Pen size (Large)	0.16	0.64	ns
Absence of food	Ref		
Presence of food	0.77	0.64	ns
Constant	-9	0.7	0.0001

ns -not significant

Table 6.15: Poisson model with frequency of biting as the outcome and pen size and food availability as predictors (overall significance of the model was P=0.53). Parameter estimates, Standard error of the coefficient (SE) and significance level are tabulated.

Predictors	Coefficient of frequency of biting	Standard error	P value
Pen size (small)	Ref		
Pen size (Large)	0.25	0.62	ns
Absence of food	Ref		
Presence of food	-0.44	0.48	ns
Constant	-7.6	0.56	0.0001

ns-not significant

6.4. DISCUSSION

6.4.1. Journey characteristics

The majority of horses transported to the slaughter plant selected for study originated from the states of Pennsylvania, Ohio, Iowa, Indiana and Kentucky in the USA and a few truckloads (one to three truckloads per week) arrived from the province of Ontario in Canada. Four of these five states of the USA (Pennsylvania, Ohio, Iowa and Kentucky) accounted for 40% of horses transported for slaughter to Canada in 2009 (Chapter 2).

New Holland auction markets of Pennsylvania, Kurtenbach Horse collection centre of Iowa and Sugarcreek auction markets of Ohio were the major sources of horses to this slaughter plant. Data regarding source of origin of slaughter horses in this chapter and chapter 2 captures only the final leg of the journey and may not be the only journey slaughter horses would have gone through before reaching the slaughter plant. Horses could have been transported from other places by horse collecting companies and transporters from different auction markets, feedlots and farms to the above mentioned source of origin.

Data in chapter 2 indicated that the slaughter plant in Québec where this study was conducted, accounted for 20% of the total number of horses slaughtered in Canada in 2009. It is possible that the closure of two slaughter plants after 2009 would have increased the percentage of horses slaughtered in this plant. The current study was therefore likely to have provided a reasonable sample of the population (horses slaughtered in Canada).

All truckloads that arrived from the USA used single deck livestock trailers connected to the truck by an articulated joint. These trailers were of the dimensions of 16 metre in length, 2.3 metre in width and 2.3 metre in height. The Canadian code for equines suggests a minimum trailer height requirement of one inch above the withers for every hand (4 inches) in wither height (NFACC, 1998). Therefore, an average quarter horse or standard bred horse of 14 hands to 16 hands (1.4 metre to 1.6 metre) will need another 14 inches to 16 inches (0.35 metre to 0.4 metre) of space above the withers. The deck height of 2.3 metres in these trailers complies with the guidelines laid out by the NFACC code (NFACC, 1998). However, Whiting and Sauder (2000) argued that the NFACC (1998) guidelines regarding deck height may not be sufficient. Some transporters had provided additional safety to horses by covering the metal sidewall of the trailers with plywood whereas others did not. There was one truckload per week which used pot bellied livestock trailers that arrived from Ontario, Canada. Horses that arrived by pot bellied truck were excluded from this study so that a difference in vehicle design would not affect the results of the study, particularly when considering the association between welfare outcomes and risk factors.

The median estimated stocking density of 349 kg/m^2 was below the maximum stocking density limit recommended by NFACC (NFACC, 1998) and Whiting (1998) for transporting loose horses. In this study, the stocking density was calculated by dividing the total live weight per load by the total area available for horses in the vehicle. The drawback with this method of stocking density calculation is the assumption that all horses in each load had equal distribution of space, which may not be the case within each truckload. The truckloads which had stallions needed separate stalls, thereby reducing the space for other horses which was not accounted for in the stocking density calculation. Therefore, it was possible that the stocking density of at least some of the loads was underestimated. Stull (2008) previously reported similar stocking densities in horses' transported for commercial purposes in the USA as reported in this study. Stocking density as a risk factor for aggression, falling down during transport and prevalence of injuries in slaughter horses was studied by some authors. Iacono *et al*. (2007) studied transport of slaughter horses and reported increased chances of horses falling down at higher stocking density (397 kg/m^2) when compared to low stocking density (221 kg/m^2). However, Stull (1999) reported that the percentage of horses injured was lower in trailers with 1.14 to 1.31 m^2 of floor area per horse than in trailers with 1.40 to 1.54 m^2 of floor area per horse. In contrast to these previous studies, in the current study, estimated stocking density did not have any association with the number of horses injured per truck.

6.4.2. Journey duration

Journey duration ranged from 6 hours to 36 hours in this study and this was within the maximum permissible limit without feed, water or rest during a single journey in Canada according to the Health of Animals Regulations (Department of Justice, 1990). The maximum permissible limit for transport of slaughter horses in the USA without feed and water is 28 hours (USDA, 2011). Horses arriving from Kentucky State had a journey duration of 36 hours which was at the maximum permissible limit. This study demonstrated that journey duration was significantly associated with an increased prevalence of injuries.

Journey duration *per se* cannot cause injuries; however, it increases the exposure time to other causal agents that trigger injuries. Causal agents for injuries during transport may be social interactions among horses such as biting (Grandin *et al.*, 1999), kicking, falling and collisions (Collins *et al.*, 2000) or improper vehicle design. The possible reason for fewer injuries in horses originating from Canada could be that they are generally transported without stoppage at borders and often travel a shorter duration than horses from the USA. Grandin *et al*. (1999) suggested that the number of horses with injuries was significantly higher in truckloads that made multiple stops than when horses came directly to the slaughter plant from auction.

Stull (1999) studied horses transported for slaughter and found significant increases in prevalence of injuries when truckloads that travelled for 27 to 30 h were compared to those having <6 h or 16 to 23 h journey duration. However, the biological plausible explanation for journey duration having an effect on prevalence of injuries could be that with longer time there were more opportunities for injury to occur from factors such as aggressive social interaction between horses, trailer interior design causing injuries and the quality of the journey e.g. poor driving or poor roads. Therefore to reduce the risk of injury, there could be a reduction in the journey duration or alternatively a management strategy to reduce social interaction by individual segregation of horses during transport. Restriction of journey duration of horses transported for commercial purposes has been debated by animal welfare experts. Cockram (2007) argued that improvements in journey quality can be as useful as restricting journey time by regulations. Some of the aspects which could be improved to increase journey quality are driving style of the driver, road conditions, vehicle design and operation, thermal conditions and ventilation, and increased fitness evaluation (Cockram, 2007).

6.4.3. Prevalence of injuries

Table 6.3 and Table 6.4 illustrate that there was a substantial prevalence of visible injuries, particularly a prevalence of 13% in slaughter horses arriving from the USA. Three previous studies on prevalence of injuries in slaughter horses after transport reported varied prevalence levels: 2% of horses with deep cut wounds, lacerations, and bite injuries (Grandin *et al.*, 1999a); 8% with abrasions and lacerations (Stull, 1999) and 28% acute injuries (Marlin *et al.*, 2011). The first two studies were undertaken in the USA and the third was performed in Europe. When compared to the previous studies which were conducted in the USA, the current study identified a higher prevalence of injuries.

One limitation in the current study was that the horses were assessed for injuries only after transport and could not be assessed before transport. Hence, it was not possible in the current study to verify that all these injuries incurred during transport alone. In addition, classification of injuries was made differently in each of these studies which hinder comparison between them. The Marlin *et al.* (2011) study which identified a higher prevalence of injuries than the current study was conducted in Europe and may not be representative of North American transport conditions. However, Marlin *et al*. (2011) were able to observe slaughter horses before and after transport and determined that the percentage of horses with contusions and excoriations (acute injuries) was higher after the journey than before.

Stull (1999) reported that among the total injuries observed in horses transported for slaughter in the USA using pot bellied and single-deck trailers, 58% of injuries were on the head region. However, in this current study when a subset of 100 horses was assessed for injury location, 12% of the total injuries were in the head region. One plausible reason for this lower percentage of injuries in the head region could be due to the fact that all horses were transported in a single-deck trailer and not in multi-deck trailers or pot-bellied trailers. Even though the prevalence of head injuries was low compared to the Stull (1999) study, the severity of the injuries in the head region was higher than the other regions, such as back, buttock, flank or legs. Most head injuries were deeper injuries (subcutaneous injuries) when compared to injuries on other locations and were bleeding, indicating acute injury obtained during transit.

Around 1% of horses had swelling around the eyes along with or without tissue injuries indicating injuries inflicted by a blow from the trailer surface. Since swelling in the head region has not been reported in other livestock transported for slaughter, horses may need special protection of the head region during transport. Head protection could be provided by padding of the trailers or increasing the height of trailers (latter not possible because of the standard bridge heights in highways). Given the fact that during this study, only single deck trailers were used, there could be multiple factors responsible for the development of these injuries in the head region apart from vehicle design. Aggression among horses in mixed groups leading to sudden head movements, difficulty in balancing during transit leading to the head striking the vehicle particularly when transported in groups and insufficient padding of the vehicle interior are three factors which need to be studied further as causative agents for head injuries.

The analysis of the location of visible injuries, carcass bruising and DT abnormal patches indicated that most injuries were on the back and buttock regions of the horses. Grogan and McDonnell (2005) studied the locations of injuries that occurred in semi-feral herd of ponies and found most injuries in buttock and trunk regions and these were associated with aggressive interactions among horses. Therefore, injuries on the back region of slaughter horses could be due to biting by conspecifics and injuries on the buttock region could be due to kicking during transport (Derungs *et al.*, 2004). Stull (1999) and Grandin *et al*. (1999) also established injury patterns in similar parts of the body of horses after transport. This supports the suggestion that kicking and biting could be an important factor in the causation of wounds. Behaviour observations (presence of biting and kicking) in the lairage also supported the view that there could be aggressive social interactions among horses that cause injuries. One obvious reason for horses behaving aggressively was because of the mixing of horses from different groups. Change in group hierarchy can be associated with injuries due to kicking and biting (Knubben *et al.*, 2008). Grandin (1999) identified that in horses transported for slaughter in the USA, the prevalence of injuries due to biting and kicking was 25%.

Prevalence of bruising (72%) in slaughter horses still remains high. Previous studies in slaughter horses in North America by Grandin *et al.* (1999) estimated a prevalence of 51%. Acute and chronic forms of bruising has also been reported to be a significant problem affecting 61% and 69% respectively of horses presented for slaughter in Europe (Marlin *et al.*, 2011). Prevalence estimation of bruising in this study was undertaken from a smaller subgroup of truckloads (100 horses from 40 truckloads) unlike the estimation of prevalence of injuries where the sample size was much larger. Therefore causal associations could not be studied because of the reduced sample size. Some of the causal factors previously identified for bruising in other livestock are transport conditions, previous stops at auction markets, aggressive social interactions among animals and improper handling (Strappini *et al*., 2009).

6.4.4. Prevalence of lameness and non ambulatory conditions

The prevalence of lameness estimated in this study was relatively low (1%). Prevalence of lameness may be associated with transport as most of these horses did not have any other chronic pre-existing conditions on examination. A similar prevalence of lameness (1.4%) was also reported by Grandin *et al*. (1999) when slaughter horses in a Texas slaughter plant were examined. It would be interesting to review the status of these lame horses in the shipper's certificate which should have recorded any pre-existing conditions. Lame horses transported loose with other horses can undergo pain and suffering because lame animals can have difficulty balancing themselves during transit.

A total of six out of the 3940 horses observed were found non-ambulatory. All non-ambulatory horses originated from the USA and were euthanized in the vehicle itself without unloading. All non-ambulatory horses travelled a long duration to reach the slaughter plant (>24 hours). Grandin *et al*. (1999) reported a non-ambulatory condition prevalence of 0.8% which was higher than the current prevalence estimation of 0.07%. Use of the USDA shipper certificate for horses coming from the USA and increased inspections on both sides of the border could have identified horses with fitness problems. Transporting unfit horses could be a possible cause for non-ambulatory conditions, however it cannot be confirmed as pre-transport examination was not performed in this study. As per Canadian Food Inspection Agency (CFIA) guidelines, every CFIA veterinarian has to provide a detailed report on the probable causes for the non-ambulatory condition of each horse that arrived non-ambulatory (CFIA, 2013). Analysis of this report could provide valuable information regarding causation. There were no dead-on-arrival horses. Dead-on-arrival horses were reported in the Alberta Farm Animal Care horse welfare report during the years 2001 to 2006 (AFAC, 2008).

6.4.5. Welfare issues related to fitness

Prevalence of horses with a body condition less than 3 (4.6% in the USA and 4.5% in Canada) and pre-existing conditions after transport (1.4% in the USA and 5.8% in Canada) could indicate that the horses transported for slaughter had long term welfare issues. Grandin *et al.* (1995) suggested that these long-term welfare issues are due to neglect on the part of horse owners rather than factors associated with transport. Canadian Food Inspection Agency (CFIA) and USDA (latter through Slaughter Horse Transport Program) are responsible for the enforcement of regulations that prohibit emaciated horses and compromised horses being transported for slaughter. Horses that are already suffering from reduced fitness, if transported could be subjected to aggressive behaviour, such as biting and kicking by other dominant horses.

A prevalence of 4.5% horses with body condition less than 3 during this study indicates that the pre-transport evaluation for fitness needs to be improved. However, the threshold set by regulatory bodies in terms of body condition could be lower than the threshold set in this study. For example, CFIA may want to restrict transport for emaciated horses of body condition less than 2 whereas; this study had a threshold of body condition less than 3. Horses with body condition less than 3 (0, 1& 2) may go in to negative energy balance when journey duration increases, thereby making them more susceptible to cold stress. Brosnahan and Paradis (2003) suggested that old (geriatric) horses tend to be emaciated (body condition score of 0 and 1).

The prevalence of horses having pre-existing conditions and horses with body condition less than 3 were higher than previous estimations by Grandin *et al.* (1999). Higher prevalence of pre-existing conditions, particularly in horses coming from Canada indicates that there may be owners, veterinarians and other stakeholders still making poor judgements regarding fitness evaluation before transporting horses.

6.4.6. Signs of dehydration

The percentages of horses that had plasma total protein concentration and plasma osmolality that exceeded the normal clinical range as classified by Kaneko *et al*. (1997) were 73 and 28%, respectively. The mean plasma total protein concentration measured in this study was 82 g/l which is above upper reference range of 79 g/l suggested by Kaneko *et al*. (1997). A mean plasma total protein concentration of 89 g/l was reported post-transport in a previous study by Stull (1999), when horses had been transported for slaughter without feed and water for 6h to 30h. The mean plasma osmolality measured in the current study was 296 mmol/kg which was below the upper reference range of 300 mmol/kg suggested by Kaneko *et al*. (1997) and Friend (2000). However, Brownlow and Hutchins (1982) after studying 100 clinically normal horses determined a mean (±sd) plasma osmolality of 282 (±6) mmol/kg. If the upper reference range suggested by Brownlow and Hutchin (1982) was considered as the cut-off value (288 mmol/kg), then a greater percentage of horses in this study would fall into the elevated plasma osmolality category. Plasma total protein (Gibbs and Friend, 2000) and plasma osmolality (Brownlow and Hutchins, 1982) are considered to be good indicators of dehydration in horses. Therefore, signs of dehydration are evident in these horses transported for slaughter as indicated by the raised plasma total protein concentration.

Provision of water during the journey could reduce the risk of dehydration in slaughter horses by compensating for the water loss due to transportation. Stull and Rodiek (2000) measured water intake of horses during transportation in summer conditions and estimated that a horse would drink approximately 23 litres of water during a 24-hour journey. Even when water was provided during transport, there was an elevation of total serum/plasma protein concentration in horses after transport compared with that before transport (Stull and Rodiek, 2000; Friend, 2000). In the current study, the duration of journey ranged from 6 to 36 hours and none of the horses were reported to have been watered during the journey.

Journey duration and season (winter or summer) significantly affected the plasma total protein concentration and place of origin significantly affected the plasma osmolality. The effect of journey duration on plasma total protein and plasma osmolality followed a similar pattern as established by Friend (2000). In the Friend (2000) study, total protein concentration was elevated above reference range after 4 hours of transport and showed a gradual increase until 28 hours of transport. Similarly the plasma osmolality was elevated above the reference range by 10 hours of transport, did not show much elevation between 10 to 18 hours then elevated steeply after 18 hours of journey.

The increase in osmolality during transport could be due to the fact that there was greater loss of water than that of solute (salt). However, osmolality may not show elevation when the loss of solute and water are in equal proportions (isotonic dehydration). The increase in total plasma proteins could be due to water loss or due to increased protein in the plasma. For example, it has been proposed that increased lymphatic flow secondary to exercise can cause elevated plasma protein concentration (Naylor *et al*., 1993). Horses may have/probably had to do work (similar to exercise) to maintain balance and to avoid other horses during the journey. As plasma total protein concentration and plasma osmolality were measured from blood collected during exsanguination (after stunning) in this current study, the values represent changes not only due to transport, but also lairage conditions and stunning. Provision of lairage did not have any effect on total plasma protein concentration and plasma osmolality which was interesting because as per Friend (2000), both these variables should have dropped below reference ranges at least in horses which are lairaged. This finding suggests that lairage conditions in the slaughter plant even with water availability may not have improved the hydration status of the horses, possibly due to dominant horses blocking the animals to drink or due to poor placement of the automatic drinkers.

Season had a significant effect on the total plasma protein concentration. Higher than normal total protein concentration in the summer was expected as horses are at increased risk of dehydration in summer due to higher rates of evaporative water loss and sweating as heat loss mechanisms (Carlson, 1983; Stull 1999). Even though sweating was not observed at the end of the journey, it could have happened during the transit. As plasma total protein concentration was raised in the majority of the horses and osmolality was not, this would raise the possibility that there was loss of solute (salt) and water in equal proportions (isotonic fluid loss) through sweating.

6.4.7. Thermal distress and respiratory infection

The respiration rate of horses transported for slaughter was significantly greater in the winter season than in the summer. This finding is interesting because other studies have reported that horses which are subjected to sub-maximal exercise and endurance exercise in the winter season showed significantly lower respiration rate when compared to the summer season (Geor *et al*., 1995; Dahl *et al*., 1986; Khon and Hinchkliff, 2010). Anecdotal observation of horses in this study also identified horses coughing and showing nasal discharge during winter. This increase in respiration rate in the winter season along with the observation of clinical signs such as coughing and nasal discharge suggests that there may have been more horses suffering from upper respiratory infections during transport in winter than summer. Higher respiration rate could also be an indication of dehydration in transported horses (Friend, 2009). However, this possibility was not supported by the results of plasma protein concentration which was significantly lower in the winter or by plasma osmolality.

Long distance transport has been associated with a reduction of the pulmonary defence mechanism in the alveolar region, resulting in more susceptibility of the transported horses to infection (Hobo *et al.*, 1997; Raidal *et al.*, 1997). However, studies to understand the specific effect of season during transport on the prevalence of respiratory infection are limited in horses. Stull and Rodiek (2002) suggested that commercial transport of horses in small groups may reduce respiratory tract infections compared to cross tied single restrained horses. In this study, horses were transported in groups (often large groups) and they showed clinical signs of respiratory infection in the winter. Increased susceptibility of horses to upper respiratory tract infections during transportation in the winter (in subzero temperatures without adequate protection from wind draft) is a potential health risk which needs to be mitigated.

Cold stress is a possibility in horses transported for slaughter as most horses transported are recreational horses reared under indoor environments and therefore they might have an elevated lower critical temperature (Morgan, 1998). However, after studying horses which are raised outdoor in Iceland, Mejdell and Boe, (2005) reported that Icelandic ponies are able to adapt normally to outdoor temperatures of up to -31° C, provided that there is sufficient quality feed and access to a shelter. Horses transported for slaughter do not get feed and water for up to 36 hours and commercial transport trailers provide less than ideal shelter facilities. There was no evidence found during this study of any indication of heat stress in horses transported. The respiration rate remained normal particularly in summer and no abnormal elevation in skin temperature was detected. There was no horse found severely depressed or sweating profusely which are salient clinical signs of heat stress exhibited by livestock (Silanikove, 2000).

6.4.8. Blood lactate concentration

During transport, horses expend energy to maintain balance in response to vehicular movements (Doherty *et al.*, 1997; Giovagnoli *et al*., 2002) and this can result in anaerobic muscle metabolism and raised serum lactate concentrations (Stull, 1999). Although in the current study, the blood lactate concentrations were above normal, there was not a significant effect of journey duration on the blood lactate concentration. The median blood lactate concentration was equivalent to the lower range of peak plasma lactate concentrations measured by Harris and Snow (1992) in horses exercised in hot and humid conditions for 6 minutes on a treadmill at 8 m/s with a 5 degree incline. The maximum concentration was equivalent to the lower range of peak values obtained from horses exercised at 10 m/s under similar conditions. After exercise, the blood lactate concentration can remain high for at least 40 minutes (Rainger *et al.*, 1994) and therefore, if the blood lactate concentration was raised by muscular exertion during transportation, it would probably still have been raised in horses that were not lairaged overnight and might also have still been raised in horses lairaged overnight (Werner and Gallo, 2008). In winter, horses that were not lairaged overnight had a higher blood lactate concentration than those that had been lairaged overnight. Changes in management between the summer and winter observation periods may have affected the handling of the horses in the lairage and reduced the blood lactate concentration. Between the summer and winter observation periods, a different worker was responsible for the care and handling of the horses in the lairage and the flooring in the lairage and stunning pen was changed to reduce slipping. Therefore, if the handling of the horses was improved by the changes undertaken, this might explain the reduction in the blood lactate concentration between the summer and winter observation periods.

Slaughter horses can experience rough handling in lairage and experience fear during stunning which could elevate muscle activity. Factors such as darkness in the lairage and stunning area, slippery flooring, noisy and improper handling can also affect the welfare of the horses (Grandin, 1996). However, the interpretation of the blood lactate concentrations in relation to pre-slaughter management is affected by the potential confounding caused by the slaughtering procedures. Werner and Gallo (2008) reported that the plasma lactate concentration increased markedly when horses were moved from the lairage and into the stunning box and it increased again after stunning. It was possible that the blood lactate concentrations in the current study were raised, at least in part, by the release of catecholamines during the slaughtering procedures, resulting in glycogenolysis (Micera *et al*., 2010), combined with muscular tension and activity after stunning (Nemec Svete *et al*., 2012).

6.4.9. Blood glucose concentration

Blood glucose concentration was measured to examine whether there was a possibility of food deprivation, physical exertion, or poor fitness associated with long journey durations causing hypoglycaemia. In this study, 50% of horses had blood glucose concentrations, which were higher than the normal clinical range and only 0.3% had a concentration below the normal range. Changes in blood glucose concentration can be influenced by feeding patterns, (Sticker *et al.*, 1995), exercise patterns (Hyyppä, 2005) and also in response to the activation of the adrenal axis. Horses transported for a short distance (1 hour) can show a significant increase in blood glucose concentration (Werner and Gallo, 2008). However, during short intense exercise, both decreases and increases have been recorded (Snow and Mackenzie, 1977; Lindholm and Stalin, 1974). Horses transported for long distances (24 hours) with or without feeding provisions also showed an increase in serum glucose (Stull and Rodiek, 2002) and cortisol concentration (Friend, 2000). In general, measurement of blood glucose in this study did not indicate feed deprivation. However, anecdotal evidence of behaviour observation indicated many of these horses were hungry in the lairage and were eating wood shavings and chewing each other's tail.

6.4.10. Behaviour and clinical signs

The majority of the horses examined after transport were behaviourally alert. This finding was contrary to the observations of Friend (2000) when horses were transported for 30 hours without water. The horses in Friend (2000)'s study showed salient behavioural signs of fatigue, such as absence of social interactions, reduced response to stimuli and depressed attitude. However, none of the horses observed in the current study showed any salient signs of severe depression, even after 36 hours of transport. Plausible reasons for this could be: firstly that the observations in this study were performed immediately after unloading under novel conditions for horses in lairage and secondly, the Friend (2000) study was done in hot and humid conditions (to understand the effects of a worst case scenario) whereas the weather conditions were milder during this study.

Behaviour associated with fear response can elicit strong muscle contractions leading to anaerobic pathways of energy production resulting in increased blood lactate concentration (Martin and Nankervis, 2002). Fear response is a factor which is of relevance in this study as the renovation work (anti-slip flooring) and management changes undertaken before winter may have played a part in the reduction of the blood lactate concentration in the winter season.

6.4.11. DT assessment for injuries

The diagnostic ability of DT (as determined by abnormal colour patches) to detect injuries and bruising was evaluated by proportion of agreement at the animal level. Comparisons between proportion of agreement of DT abnormal patches and carcass bruising showed significant difference. The reduced proportion of agreement of DT abnormal patches with that of carcass bruising could be because abnormal colour patches in DT can happen not only due to bruising, but also by close contact with other horses or hard objects which might create minor hyperaemic areas. The majority of the high skin temperature patches that were in the back region might have been due to bite injuries which may be superficial and may not have resulted in bruising. Abnormal patches in the buttock region could be due to kick injuries or contact with the vehicle or other horses. Leg regions are also prone to kick injuries. Abnormal colour patches in the head region could be largely due to bruising and injury. Flank region abnormalities could be because of bites or contact with other horses.

6.4.12. Conclusion

The welfare issues identified in Canada were injuries (visible skin injuries and bruising), non-ambulatory conditions and signs of dehydration. The majority of the slaughter horses slaughtered in this slaughter plant originated from the USA and were transported using a single-deck trailer. There had been some improvements in the welfare status of horses that are transported for slaughter when compared to previous studies. The important ones were; there were no dead-on-arrivals, reduction in non-ambulatory animals and a reduction in the prevalence of head injuries. Reduction in blood lactate concentration in response to possible improvement in managemental conditions in the slaughter plant is a significant finding which can help slaughter plant management to allocate necessary resources to improve management conditions in the lairage. The welfare issues identified by the current study were: injuries, possible signs of dehydration, transport of horses with fitness problem (pre-existing conditions and debility) and a few horses becoming non-ambulatory. Evidence of aggressive behaviour such as kicking and biting was observed in the lairage. Journey duration, season (summer or winter), and mixing of unfamiliar horses during transport are risk factors associated with the welfare outcomes measured. Reduced journey duration, segregation of horses during transport and improved handling and management of horses in the lairage and during transport are likely to improve the welfare of horses.

6.4.13. Recommendations

This study has elucidated that there are some practical improvements that can be undertaken to improve the welfare of horses transported for slaughter in Canada. Journey duration can be reduced, by approving more slaughter plants for horse slaughter particularly in USA, thereby reducing the risk of injuries and dehydration. Horses can be segregated by provision of individual space (separating each animal with solid dividers) during transport to reduce injuries due to biting and kicking. Padded trailers may help reduce injuries in the head region. Increase pre-transport veterinary evaluations can reduce unfit animals being transported. Horses should not be lairaged for long hours or days, because the behaviour assessment showed that aggression among conspecifics particularly when mixed grouping is unavoidable in the lairage.

Pre-transport and post transport evaluation for welfare should be an integral part of health management system and the data has to be monitored continuously. Detailed classification of injuries as similar to this study will help identify associated risk factors. Plasma or serum total protein concentration and blood lactate concentration and should be performed regularly to assess dehydration status of horses arriving for slaughter and high metabolic activity.

6.4.14. Future research

Aggressive interaction between horses during transport was identified as one of the causal agents for the prevalence of injuries in horses transported for slaughter. Bite wounds on the back region of the horses and kick injuries in the leg and buttock region were clearly evident in this study. Future research is needed on these specific aggressive behaviours of horses during transport particularly when they are transported in mixed loose groups. If future research demonstrates that horses of mixed origin cannot be transported without exhibiting these behaviours, then it will be prudent to separate individual horses during transport of horses for slaughter.

The relationship between injuries in the head region particularly around the eye and interior designing of the vehicle need to be studied further.

Anecdotal evidence of respiratory infection had been noted particularly when horses were transported during winter. Future research should focus on identification of the component causes for respiratory infection in winter and ways of mitigating that risk.

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CHAPTER 7

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

7.1. GENERAL DISCUSSION

7.1.1. Introduction

Welfare of horses transported for slaughter is a growing concern in several countries including the United States of America (USA), Canada and Iceland. In Canada and the USA, unwanted horses at the end of their productive use for recreation or sport may be transported to slaughter plants for slaughter. Animal welfare groups such as the Humane Society International uphold the view that slaughter of unwanted horses for human consumption increases the chances of suffering due to long journey duration and poor slaughter management procedures, such as inadequate stunning (Humane Society International, 2012). On the other hand, the American Veterinary Medical Association (AVMA) advocates the view that slaughter of unwanted horses at a slaughter plant is better for the welfare of horses compared to the risk of neglect and abandonment by their owners (AVMA, 2013). In Iceland, unlike North America, Icelandic ponies are farmed for slaughter and there is no evidence that unwanted horses are a problem there.

This study of horses transported for slaughter in Canada and Iceland is one of the first to assess welfare implications of handling and transport of horses leading up to slaughter. Outcomes of this study may have significant consequences in North America especially given that the USA stopped the slaughter of horses for human consumption in that country in 2007, and most horses intended for slaughter from the USA are transported to Canada. In Canada, export of horse meat is approximately a 100 million dollar industry, but public concern about transport and slaughter of horses has been growing. In Iceland and the rest of Europe, interest in the slaughter of horses for human consumption has become an issue of public debate following the identification of horse meat in meat products that were not sold as containing horse meat.

Stull (2012) opined that transport of horses from the USA to Canada and Mexico compromised the welfare of horses through increased journey duration and possibly by low standards of handling and stunning practices in slaughter plants. Data from Chapter 2 (shipper certificate data) and Chapter 6 (Québec slaughter plant data) showed that a significant proportion of horses slaughtered in Canadian slaughter plants continue to originate from the USA. It seems likely that stoppage of horse slaughter for human consumption in the USA has increased the journey duration of transport for slaughter horses as indicated by the data in Chapter 2. This increase in journey duration could be due to increased distances between the origin of the journey and the slaughter plant combined with waiting times for border inspections. The effect of journey characteristics on various aspects of the health and welfare of the horses was evaluated in this study. However, an objective evaluation of handling and stunning practices in Canadian slaughter plants could not be performed in this study due to workplace sensitivity and approval issues.

7.1.2. Objectives

Establishing a welfare assessment protocol for horses transported for slaughter was an important objective of this study. Welfare assessment variables for the protocol were chosen after extensive literature review. In the Canadian slaughter plant, assessment of welfare was accomplished by observing horses at four critical points: observation while unloading of horses from the truck, observation during lairage, measurement of physiological values in blood collected during exsanguination and observation of carcasses for bruising. In Iceland, an additional pre-transport examination was performed when the horses were loaded on to the truck.

After identification of welfare issues, the second objective was to identify the risk factors associated with each of the welfare issues. For example, journey duration was significantly associated with the number of animals injured in each truckload. In Canada, the location of injuries and their severity indicated that injuries may be associated with aggressive behaviour, such as biting and kicking among horses. Injuries that are not visible ante-mortem (i.e. bruising) were assessed from the carcass post-mortem. Identification of the welfare issues and their respective risk factors in this study should allow the development of mitigation strategies and hence improve welfare.

Some of the recommendations from this study can feed into the formulation of guidelines for best practices to avoid unnecessary suffering of horses transported for slaughter. For example, counting the number of horses injured per truckload for a predetermined number of truckloads in a month or any other defined time period could be useful in identifying the factors causing these injuries. These measurements can be obtained by non-professional staff with some training who can report back to the CFIA veterinarian. Capturing welfare issues such as injuries in a systematic manner can help identify risk factors, thereby enabling us to improve welfare by mitigating risk factors.

7.1.3. Welfare assessment methodology

Inspections and regulations to improve the welfare of horses intended for slaughter are effective and useful only if good welfare assessment protocols are developed and implemented. The welfare assessment protocol used in this study to identify pertinent welfare issues was simple, practical, and can be performed in most slaughter plant lairage conditions. Most of the welfare assessment variables that are part of the protocol are animal based variables such as direct observation of the animal for non-ambulatory condition, injuries and body condition evaluation. Clinical observations of the animal, such as respiration rate and lameness assessments used were easy to measure without handling animals. Physiological assessment variables were measured in blood collected during exsanguination, and hence no additional handling of horses was required. Management based variables such as journey duration and stocking density, and environmental based variables such as ambient temperature were part of the protocol.

Fitness evaluation for transportation of horses was assessed by measuring the prevalence of horses with body condition less than 3 (on a scale of 5) and the prevalence of pre-existing conditions. As both these variables indicate chronic issues, they are relevant to fitness assessment for transportation.

Signs of dehydration were assessed by measuring total plasma protein concentration, osmolality and packed cell volume. These physiological variables provided only an indirect method of measuring dehydration; however, their usefulness has been established by previous welfare assessment studies (Friend, 2000).

Increased muscle activity was assessed using blood lactate concentration and the effect of feed restriction was assessed by measuring blood glucose concentrations. Blood lactate concentration provided useful information regarding anaerobic muscle activity during transport and subsequent management and hence could be used in a future welfare assessment protocol as a variable to assess the amount of muscle activity. The absence of blood glucose concentration measurements indicative of hypoglycaemia indicated that the horses were not in metabolic collapse associated with prolonged feed restriction during transport.

Previous studies have pointed out that some of the risk factors for welfare can occur during lairage (Sandström 2009; Werner and Gallo, 2008). In this study space allowance available for horses, food and water provision, biting and kicking behaviour and feeding and drinking behaviour among horses were observed in the lairage (Chapter 6). The selection criterion for choosing to study these factors was dependent on the feasibility for observation. An interesting finding in the Canadian slaughter plant lairage was the observation of biting and kicking among mixed groups of horses, which might increase the risk of injuries. However, other observations, such as feeding and drinking behaviour, which may have helped in the evaluation of hunger and thirst could not be undertaken immediately after the horses were unloaded. Therefore, the latency by the horses to eat and drink after arrival could not be recorded.

Effectiveness of stunning process (percentage of horses made unconscious immediately after stunning) has a direct effect on the welfare of horses slaughtered for human consumption. The stunning process was assessed by using behavioural variables indicative of consciousness. Assessment regarding the stunning process was performed only in Iceland and not in Canada. Due to the difficulties in obtaining access to an equine slaughter facility in Canada, the controversy over the effectiveness of stunning was considered to be too sensitive and the studies in Canada concentrated on the main objectives related to transportation.

7.1.4. Prevalence of welfare issues

The welfare issues identified in Canada were injuries (visible skin injuries and bruising), non-ambulatory conditions, signs of dehydration and respiratory infections. In Iceland, injuries and signs of dehydration were the two welfare issues identified, however these were found at lower level than those recorded in Canada. Most of these welfare issues identified by this study were also identified by previous studies in North America and Europe although the prevalence levels varied amongst these and the current study.

Longitudinal comparison of some aspects of welfare with previous work indicates that there may have been improvements in the welfare of horses transported for slaughter in North America. In 1998 and 1999, when Grandin *et al*. (1999a; 1999b) observed 1,008 horses intended for slaughter after transportation in the USA, they recorded four dead-on-arrival horses (prevalence of 0.4%). By 2008, the Alberta Farm Animal Care (2008) reported results showing a yearly reduction of dead-on-arrival horses. In 2001, 53 horses were declared dead-on-arrival out of the 66,255 horses transported for slaughter in

Canada (prevalence of 0.08%). The number of dead-on-arrival horses reduced gradually to only nine horses in 2006 (prevalence of 0.02%). The 2009 shipper certificate study (Chapter 2) found a prevalence of <0.01% dead-on-arrivals horses, and the data collected in 2011 and 2012 in one of the slaughter plants in Canada (Chapter 6) did not find any dead-on-arrival horses. One possible reason for the reduction in dead-on-arrivals could be the introduction of fitness evaluation of horses prior to transport in the USA (shipper certificate). Other possible reasons could be due to increased inspection by the USDA, removal of non-ambulatory animals after euthanasia and removal of dead animals during transport in the USA (Department of Agriculture, Animal and Plant Health Inspection Service, 2008) and also inspection by CFIA officials at the slaughter plant

Despite a reduction in prevalence of dead-on-arrival horses in Canadian slaughter plants, the prevalence of injuries on the surface of the body of transported horses still remains high (prevalence of 12.8% in horses transported from the USA). Another finding which highlights the magnitude of injury as a welfare problem is the prevalence of head injuries (4%). Head injuries were mostly deep with bleeding and swellings around the eye region. A previous study by Stull (1999) had indicated that a double-deck trailer design could be the causative agent for head injuries and hence restrictive regulations were introduced in the USA to reduce head injuries. Head injuries were not expected in this study as the use of double-deck trailers should have been discontinued for the transport of slaughter horses from the USA to Canada (Chapter 6). In view of the fact that abolition of double-deck trailers has not eliminated the prevalence of head injuries, other risk factors such as vehicle interior design, aggressive behaviour among horses during transport and fatigue related to transport need to be studied to identify probable causative factors.

The prevalence of bruising as estimated in Chapter 6 was 72%, higher than the previous study in North America by Grandin *et al*. (1999b). Bruising in Canadian slaughter plants needs particular attention by the slaughter plant managers and CFIA officials because of the size and severity of the bruises identified. Apart from economic loss, bruising is a significant welfare issue because it is caused by blunt trauma and is associated with pain and suffering (Strappini *et al*. 2009). Bruising was also a welfare issue among Icelandic slaughter horses, particularly in the adult horses. However, the magnitude of bruising in Icelandic slaughter horses in terms of size and severity was much lower than in Canada. The probable causes for bruising in both countries (Canada and Iceland) could be different. The horses in Canada had larger bruises and more bruises all over the body. Probable causes for these bruise could be kicks and bites from other horses and contact with vehicle walls. However, in Iceland, anecdotal observation indicated that bruising was in the gluteal region indicating handling as the probable cause. Due to poor design of lairage facilities in Iceland, horses needed intense handling while unloading after transport and during movement to slaughter.

The prevalence of bruising in the Canadian slaughter plant was higher than visible injuries, which indicates that injuries during transport might be underestimated if only ante-mortem visible injuries are assessed. However, it is hard to prove that high prevalence of bruising in the Canadian slaughter plant was associated with transport alone. Some of the injuries could have occurred in the auction market, before transport or even during handling at the slaughter plant and during slaughter.

The prevalence of non-ambulatory horses (0.07%) after transportation from the USA to Canadian slaughter plant is a serious but infrequent welfare problem. Horses transported in a non-ambulatory condition may be trampled upon by other horses. According to CFIA's compromised animal policy, non-ambulatory horses should undergo CFIA official's inspection and a written ante-mortem veterinary report should be generated (CFIA 2013). Further analysis of this ante-mortem veterinary report may provide more information regarding causes for non-ambulatory conditions as perceived by the CFIA officials. On the other hand, there were no horses in a non-ambulatory condition in loads that originated from Canada, which indicates a good trend in terms of welfare. No non-ambulatory horses were observed in Iceland.

Signs of dehydration were identified in slaughter horses at both the Canadian slaughter plant and Icelandic slaughter plant. The Canadian slaughter plant had automatic water troughs available in the lairage for horses and hence during lairage there was a possibility for some recovery from dehydration incurred due to the long journey to the slaughter plant without access to water. Therefore, dehydration levels could have been higher if assessed immediately after transport. In Iceland, where both adults and foals were slaughtered for human consumption, adults showed more signs of dehydration than foals, possibly because the adult horses were mainly lactating mares and had less access to water in the lairage than the horses in Canada. Comparing the risk factors for dehydration in Iceland and Canada indicates that the probable causes for dehydration in both countries could be different because the duration that the horses were transported without water and management conditions in the lairage varied between countries.

Signs indicative of respiratory tract infection in horses transported during winter months from the USA to Canada were another welfare issue identified by this study. Apart from anecdotal evidence of coughing and nasal discharge, respiration rate was higher in winter than summer. Factors such as subclinical infections before the start of transport or infections acquired in auction market are probable risk factors. The possibility of upper respiratory tract infections in horses transported during winter could be one of the reasons for increased respiration rate, but this needs to be investigated further.

Signs of high anaerobic muscle activity were identified from the blood lactate measurements of slaughter horses that were transported and lairaged in Canada and Iceland. From the information obtained in Chapter 3 and Chapter 6, the likely explanation for the increase in blood lactate concentration above the reference clinical range could be the increased anaerobic muscle activity during the stunning procedure (e.g. struggling in stunning pen and post-mortem movement or muscle spasms), because journey duration did not have any significant effect on blood lactate concentration (neither in Iceland nor Canada). However, this interpretation regarding a non-significant effect of transport on blood lactate concentration needs to be cautious, because the measurement of blood lactate concentration was performed using blood collected during exsanguination and not immediately after transport. The half life of blood lactate is around 30 minutes and hence any indication of extreme muscle activity during transport would have been mitigated during lairage or any other rest periods (e.g. stationary vehicle)

Severe fatigue as reported by Friend (2000) after 27 hours of transport was not observed in slaughter horses in Canada, even after 36 hours of transport. Friend (2000) indicated that after 27 hours of transport, horses became noticeably quiet and had a tucked up appearance of the abdomen, but this was not noticed in this study during observations in the lairage after transport. Plausible reasons for horses not showing depressed behaviour in this study could be because of the novel surroundings of the slaughter plant, mixing of unfamiliar horses in the lairage after unloading and lower ambient temperature during the journeys compared with those undertaken in the Friend (2000) study.

7.1.5. Fitness for transportation

The USDA introduced a shipper certificate as a legal document for fitness evaluation of slaughter horses pre-transportation to improve the welfare of horses that are transported from the USA to Canada and Mexico. However, horses intended for slaughter originating from within Canada currently do not need to have any fitness evaluation documentation. The Health of Animals Regulations of Canada prescribes that unfit animals cannot be transported. Despite the fact that horses which originated in Canada did not undergo fitness evaluation documentation, the prevalence of horses with body condition less than 3 and those that became non-ambulatory during transportation were lower than those horses transported from the USA. On the other hand, there was increased prevalence of pre-existing conditions in horses that originated from inside Canada compared with those from the USA (Chapter 6). A pre-existing condition was defined as the presence of a chronic medical condition (e.g. granulating wounds and arthritis). According to a previous study by Grandin *et al*. (1999b) pre-existing conditions made the horses more prone to welfare issues such as injuries when transported for slaughter. The Health of Animals Regulation (Part XII) states that animals which are afflicted with “illness and injury cannot be transported”. Chronic wounds and chronic arthritic joints (pre-existing conditions) could fall under the category of injury as per the regulation and hence pre-transport fitness evaluation in Canada needs to be implemented.

7.1.6. Journey characteristics

Vehicle types

Horses intended for slaughter originating from the USA and transported to Canadian slaughter plants used a single-deck articulated vehicle for transportation. Horses originating from within Canada used both a single-deck articulated vehicle and also a double-deck articulated vehicle. However, when a double-deck trailer was used for transportation, only one deck of the trailer was loaded with horses and the other deck was not used, providing more head room for the horses than when both decks of a double-deck trailer are used. Horses transported in a double-deck articulated vehicle were not included in this study because the numbers of these consignments were negligible.

In Iceland, a single-deck non-articulated livestock trailer was used for most transport of horses. The dimensions of length and breadth for this vehicle were smaller than those in the vehicles used in Canada. The use of a single type of vehicle limited the comparisons between welfare outcomes of different vehicle designs but permitted greater replication to study other factors. Occasionally, owners used their own horse floats to deliver horses to the slaughter plant. These horses transported by owners themselves were not included in this study. However, this accounted for a negligible percentage of consignments. Horses in Iceland, did not suffer from any of the head injuries seen in the horses transported to Canadian slaughter plants. This could be because of the very short journey duration and also could be because the height of these Icelandic horses was much shorter than North American horses, which allowed more head room while in transit.

Source of origin of horses and journey duration

In the Canadian slaughter plants, a high percentage of horses slaughtered for human consumption originated from the USA. Transport of horses across national borders involves issues related to inspection at the border that increases journey duration. Apart from country of origin, the sourcing of horses - whether it is from an auction market, feedlot or horse collection centre, can also have an impact on welfare. Horses that arrived at the slaughter plant in Québec, which were studied for welfare assessment in Chapter 6, originated only from auction market in the USA and Canada. However, Chapter 2 indicated that some percentage of horses can originate from feedlots. This finding suggests that even though the slaughter plant in Québec had all horses originating from auction markets, other slaughter plants in Canada may receive horses from feedlots. On the other hand, in Iceland, the origin of journey was always individual farms where slaughter is used as means of stock management.

Journey duration for horses coming to the slaughter plant in Québec ranged from 8 to 36 hours (Chapter 6)**.** However, the retrospective study of 2009 shipper certificates (Chapter 2) indicated that a considerable portion of consignments originating from the USA were transported for longer than 36 hours. This change in scenario for reduction in journey duration between 2009 and 2011 may be because of increased inspection and better compliance by the transporters. Alternatively, it may be an artefact caused by the different methods of data collection. In Iceland, journey duration was short and journey distance was less than 200 km because there are eight slaughter plants spread across Iceland.

Journey duration of horses transported from the USA to Canadian slaughter plants requires more investigation because the primary aim of the shipper certificate was to evaluate fitness of horses transported and not the journey duration. As a result, it is possible that the CFIA officials are not recording the journey duration as per a clear definition of start and end of journey in the shipper certificate. If so, the shipper certificate needs to be upgraded to capture the journey duration more accurately. For example, in Europe, there are "Animal transport Certificates" and "journey logs" which allow transporters to provide detailed information about journey duration in order to comply with Article 4 and Article 6 of European Commission Regulation (Council Regulation (EC), 2005).

7.1.7. Relationship between journey characteristics and welfare assessment variables

Journey duration and injuries

In Canada, journey duration had a significant association with the prevalence of visible injuries. Aggressive behaviour among horses when transported in groups, and muscle fatigue due to transport which reduces the horses' ability to respond to vehicle movements and other horses are two factors which can cause injuries when journey duration increases. In Iceland, journey duration did not show any association with bruising probably because the journey durations in Iceland are generally short. However, adult horses in Iceland were more prone to injuries probably due to increased handling of these horses in the lairage and during stunning. The implications of these findings are that there are two ways the risk factors for injuries can be mitigated during the transport of slaughter horses. One way is to reduce the journey duration. Another way is to improve the journey quality by reducing the potential for aggression among horses transported and also by improving the interior design of the vehicles.

Journey duration and blood lactate concentration

Journey duration did not have a significant association with blood lactate concentration in both the studies undertaken -in Canada (Chapter 6; 6-36 hours), and in Iceland (Chapter 3; 20 to 185 minutes). This finding is contrary to some previous studies, which had shown a significant increase in blood lactate concentration with increase in journey duration. However, other factors such as lairage duration, handling in lairage, season and stocking density in the lairage showed a significant association with blood lactate concentration. Biologically, blood lactate concentration increases with anaerobic exercise and hence indicates that muscle activity of high intensity could be happening after the transport in the lairage or during stunning. The implication of this finding is that horses should be handled better during the lairage and during stunning. If the horses are managed in such a way that they are calm in the lairage and stunning pen, fear-related behaviour and the associated muscle activity will also be reduced.

Journey duration and plasma total protein concentration

Journey duration did have an association with total protein concentration in the Canadian study supporting the findings of previous studies. In winter, there was significant reduction in plasma total protein concentration when compared to summer, despite longer journey durations. This finding can be explained biologically in view of the fact that horses will need less water in winter because evaporative loss and sweating would be reduced. Another interesting observation was the absence of recovery from dehydration of horses with high total protein concentration which Friend (2000) noticed after horses had been given access to water after transportation. This indicates that the water troughs in the lairage may not be fully effective in alleviating the dehydration suffered by the horses during transport.

Journey duration and plasma osmolality

Journey duration had a borderline significant association with plasma osmolality when Canadian slaughter horses were studied in Chapter 6. Friend (2000) also established that horses undergo hypertonic dehydration during transportation due to water loss leading to increased plasma osmolality. In the Friend (2000) study, horses showed a higher than normal range of osmolality after 8 hours of transport and very high elevation of osmolality (330 mmol/kg) after 20 hours of transportation, even when the horses were watered during transport. Horses transported for slaughter to Canada in this study did not get feed or water during transport and hence the osmolality would be expected to be higher. However, the upper quartile of osmolality did not rise above 310 mmol/kg. The probable reason could be because horses were provided with water in the lairage that could have had a lowering effect on the plasma osmolality.

7.1.8. Significance of research work

This study provided a bench mark for baseline estimates on the prevalence of welfare issues such as injuries, dead-on-arrival horses and non-ambulatory conditions after transport for the population of horses slaughtered for human consumption in Canada and Iceland. Although infrequent, the presence of horses with non-ambulatory conditions in Canadian slaughter plants indicates that some serious welfare issues persist. However, there was no occurrence of dead-on-arrival horses, indicating an improvement in welfare status because previous studies had reported this welfare issue.

Studying welfare issues associated with transport of horses for slaughter in two geographic locations (Canada and Iceland) helped to make comparative interpretations regarding risk factors such as journey duration. For example, the blood lactate concentration was elevated above reference range in horses transported for short journey duration in Iceland (Chapter 3) and also after relatively long journey duration in Canada (Chapter 6). This indicates that factors other than journey duration may have had an effect on the blood lactate concentration of slaughter horses. Another example was; when journey duration was shorter (<1 to 3 hours) as observed in Iceland, the prevalence of visible injuries was negligible, and when the journey duration was higher (8 to 36 hours) as observed in Canadian slaughter plants, the prevalence of injuries was high.

A welfare assessment protocol to assess welfare of horses at the end of transport on arrival at the slaughterplant similar to the one used in this study would be beneficial to improve welfare in Iceland and Canada. Currently, the main focus of the slaughter officials is to reduce or stop contamination of meat for human consumption with infective agents and chemicals and to identify non-ambulatory animals, which need special handling (CFIA, 2013a). Ante-mortem inspection focussing on improving animal welfare aspects such as injuries could be added because the Health of Animals Regulations of Canada prescribes that animals should not be injured during transport. Any animal-based inspection or assessment focused on improving the welfare of horses such as quantifying injuries after transport will be a step forward to improve welfare both in Canada and in Iceland.

Understanding the associations between injuries and risk factors such as journey duration and aggressive behaviour between horses is another significant finding. Interventions to mitigate injury could be achieved by reducing the journey duration and providing separation devices during transport to reduce biting and kicking. European Food Safety Authority had previously suggested separation of individual horses during transport for slaughter (European Food Safety Authority, 2011). However, after evaluating the implementation of this recommendation, others (World Horse Welfare, 2008) remain concerned that without appropriate separation, there is an increased risk of injury. After a particular time period following implementation of these interventions, welfare assessments can be carried out again and comparisons made with bench mark estimates to evaluate effectiveness of the interventions.

The very high prevalence of bruising in horses slaughtered in Canada makes the identification of risk factors associated with bruising an important priority to improve welfare because bruising is associated with pain and suffering. Detection of bruising during post-mortem carcass evaluation complicates the identification of risk factors associated with bruising because the bruising identified on the carcass does not only relate to transport aspects. Hence, detection of bruising ante-mortem using Digital infrared thermography (DT) as a potential diagnostic tool can help slaughter plant personnel or CFIA employees to identify horses that have suffered from bruising obtained during transport and prior to it. To identify bruising ante-mortem, a novel methodology using DT was developed. The advantage of this method is that bruising could be detected ante-mortem, by taking DT pictures before (not obtained in this study) and after transport. By this means, the incidence of bruising and the risk factors associated can be evaluated more effectively. The sensitivity (proportion of true positives correctly identified) of the methodology developed was modest and needs further refinement to optimise the techniques. The results obtained in this study suggested that increasing the time available after transportation for the skin temperature of horses to come to equilibrium with the ambient temperature in lairage, using a better-quality infra red camera and only taking images when the lairage ambient temperature is close to the thermo-neutral zone of horses would increase the sensitivity of this methodology. The research conducted on the topic of DT to identify bruising ante-mortem could be an aid to improve the welfare of horses that are transported for slaughter for human consumption.

7.1.9. Pros and cons of the research approaches used

To achieve the objective of identifying of welfare issues and associated risk factors, a number of different research approaches were used in this study. A retrospective cross-sectional research approach was used in Chapter 2, to analyse the shipper certificate data from 2009. To identify welfare issues in horses transported in Iceland, (Chapter 3) Canada and the USA (Chapter 6), a prospective longitudinal (cohort) study was used in Iceland and a cross-sectional approach was used in Canada. In the Canadian study, observation of horses could only be performed after transport and pre-transport observation was not possible. However, in the Iceland study, horses could be observed pre-transport and post-transport; thereby findings such as skin injuries are causally associated with the transport.

A cross sectional study may not be as effective as a cohort or experimental study in identifying causation factors for welfare issues as it does not include the time when the risk factor came in to play. Hence, additional cohort studies which focus on the risk factors associated with specific welfare issues are recommended to establish causation particularly in Canada. For example, horses can potentially acquire injuries at an auction market prior to transport. Undertaking cohort studies in Canada by observing horses pre-transport and post-transport would provide more valid information than cross sectional studies for identification of risk factors, which affect welfare during transport. A cohort study could not be undertaken in Canada due to logistic reasons.

The cross sectional observational research approach was used effectively in Chapter 6 (Canada study) to capture multiple risk factors for multiple welfare outcomes. Moreover, some welfare outcome variables (particularly physiological variables) measured using observational research approach behaved similar to welfare outcome variables measured in experimental studies on equine transport. For example, the effect of journey duration on plasma total protein concentration and plasma osmolality resembled the experimental results of Friend (2000) . This comparison is useful because an experimental research approach provided stronger evidence of causation than an observational research approach, while the latter provides a better estimate of what happens in the "real world". In an observational cross-sectional study, it is difficult to determine the timeline when the risk factor the researcher is interested in began, whereas in an experimental study the risk factor is introduced by the researcher and hence is well aware of the time line. In addition, results obtained from experimental studies are not affected by confounding factors due to the researcher having control over the selection of study subjects, while observational studies allow for the simultaneous evaluation of multiple risk factors.

Behaviour assessment for aggressive interaction was possible in the Canadian slaughter plant because the lairage was built as per OIE standards. However, this was not the case in Iceland because the stocking density was extremely high in the lairage and there were not enough lighting arrangements to do behavioural observation**.**

To improve the welfare assessment methodology used for horses during transport and during the slaughter procedure, some additional variables could be added in future studies. These include information regarding interior design of the vehicle (particularly to detect any association with head injuries), noise levels in the lairage, time spent in the stunning box and time taken between stunning and sticking (exsanguination). These factors have been associated with reduced welfare in previous studies. However, variables that represent these factors could not be included in this study due to logistical reasons.

Both studies, one conducted in Canada (Chapter 6), and another conducted in Iceland (Chapter 3) were designed to obtain a representative sample of the total horses slaughtered in each country. Information obtained from analysis of one-year data of shipper certificates obtained from the USDA regarding journey characteristics and animal characteristics (Chapter 2) indicated that the sample size studied in Chapter 6 was representative of the population of horses slaughtered in Canada. The Québec slaughter plant, where data collection was conducted, is one of four slaughter plants in Canada that slaughter a high number of horses. However, there could be minor differences in the management of slaughter horses by different slaughter plants in Canada (e.g. some obtained horses from feedlot or auction centres or both). Overall, the evidence obtained from Chapter 6 regarding estimated prevalence of welfare issues and associated risk factors is pertinent to the target population of horses transported for slaughter in Canada. However, using coat markings to identify sampled horses for detailed assessment has a potential for bias because standardbred horses tend to have no markings on the body and quarter horses have more markings, making the later likely to be selected more often. In Iceland, approximately 2% of the horses slaughtered per year were studied in one of the biggest slaughter plants so that a good representative population was captured.

Blood was collected at exsanguination to evaluate whether blood samples collected without the need for restraint and handling, and analysed using basic on-site assay procedures could provide useful information on blood chemistry to assist in the assessment of the welfare status of the horses after transport to the slaughter plant. Routine handling of horses within a slaughter plant lairage for blood sampling is potentially dangerous and interferes with the commercial operations. However, there are a number of difficulties with using blood collected at exsanguination to provide information on the status of the horses after transportation. Many of the variables studied would likely change during any lairage as a result of rest after transportation, the ingestion of food and water, interactions between horses and the environmental conditions within the lairage. In addition, the movement of the horses from the lairage area, confinement in the stunning box, the act of stunning, and post-stunning limb movements affect many of the variables studied.

7.2. LIMITATIONS OF THIS STUDY

In this study, the welfare of horses slaughtered in Canada was assessed post-transport. Pre-transport welfare assessment could not be performed in Canada due to logistical reasons, and hence welfare issues identified cannot be directly linked causally to the transport aspect alone because management of slaughter horses involve other aspects such as horses spending time in auction centres or feedlot. Hence, the welfare outcome estimates from this study provide only prevalence and not incidence.

Information regarding journey duration in Chapter 2 and Chapter 6 may have included rest periods that the driver took in-between journey stages and border inspection stops, which could not be quantified in this study. There was a possibility that these horses would have undergone additional transportation event before reaching the declared origin of journey (auction centre) which was not captured in this study**.**

All horses studied in Canada were transported from auction markets to the Québec slaughter plant. From the data provided in Chapter 2, there was evidence to indicate that a sizable population of horses that reach slaughter plants in Canada originate from feedlots, which were not represented in this study.

The time available to the assessor for assessment of horses in the lairage was limited for some consignments. Since horses were not handled during assessment and were observed from a distance, visualising the entire surface of the horses for injuries was difficult in some instances. This could have led to underestimation of prevalence. Sandström (2009) experienced similar problems when developing a monitoring system for the assessment of cattle welfare in abattoirs.

7.3. RECOMMENDED FUTURE DIRECTIONS

Two broader questions that arise from this study are: 1) “can slaughter horses be transported to slaughter plants with no injuries, dehydration and fatigue? and 2) can horses be slaughtered with minimal welfare implications occurring during slaughter management process such as lairage and stunning? This study indicates that welfare issues such as injuries and dehydration can be mitigated by eliminating risk factors such as prolonged journey duration and aggressive behaviour between horses during transport. Additional studies are necessary to answer the second question regarding welfare implication associated with slaughter management of horses.

The recommendations from this study are for the four stakeholders involved in horse slaughter management: Health of Animals Regulation implementing agencies such as CFIA, the horse slaughter industry, policy makers and the animal welfare researchers.

Recommendations for implementing agencies such as CFIA and the horse slaughter industry who believe in self-regulation to improve welfare are:

- A shipper certificate should be introduced for horses of Canadian origin similar to horses originating from the USA as a self-regulating mechanism to document fitness issues. This could possibly reduce pre-existing conditions, which had a high prevalence in Canadian horses.

- Journey duration and rest periods during transportation have to be recorded in the shipper certificate. Vehicle stationary periods such as waiting periods at the border for inspection, and driver resting time should also be recorded along with intentional provision of rest for animals.
- There was a high prevalence of injuries identified in this study, even though the Health of Animal Regulations says that "No person shall load or unload, or cause to be loaded or unloaded, an animal in a way likely to cause injury or undue suffering to it". Welfare assessments to quantify injuries after transportation should be incorporated as a regular slaughter plant procedure. CFIA officials should actively look for injuries associated with transport and reporting systems have to be developed to capture injuries during lairage.
- Regulatory agencies should support and facilitate full disclosure and cooperation for research of this type in order meet the needs of society for optimal animal welfare.

Recommendation for policy makers are:

- Journey duration limits (currently up to 36 hours transport without food and water) could be lowered in the Canadian Health of Animals Regulation.

Recommendation for animal welfare researchers are:

- Behavioural studies need to be conducted during transport to isolate risk factors associated with aggressive behaviours such as biting and kicking in horses transported in groups.

- Associations between respiratory infections in slaughter horses and season (particularly winter season) need to be studied further.
- Associations between vehicle interior designs and head injuries (particularly around the eye region) should be studied further.

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